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- (54) NOVEL METHOD FOR PREPARING
 METABOLITES OF ATORVASTATIN USING
 BACTERIAL CYTOCHROME P450 AND
 COMPOSITION THEREFOR
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(57) ABSTRACT

Provided is a novel method for preparing metabolites of atorvastatin using bacterial cytochrome P450, and a composition therefor, and more particularly, a composition for preparing 2-hydroxylated product of 4-hydroxylated product from atorvastatin including bacterial cytochrome P 450 BM3 (CYP102A1), CYP102A1 mutants, and chimeras derived from the CYP102A1 mutants, a kit therefor, and a method for preparing thereof.

[Fig. 1]

1	MTTKEMPQPKTFGELKNLPLLNTDKPVQALMKTADELGETFKFEAPGRVTRYLSSQRLTK
61	EACDESRFDKNESQALKFVRDFAGDGLFTSWTHEKNWKKABNILLPSFSQQAMKGYHAMM
121	VDIAVQLVQKWERLNADEHTEVPEIMTRLTLDTIGLCGFYYRFNSFYRDQPHPFTTSMVR
181	ALDEAMNKLQRANPDDPAYDENKRQFQEDIKYMNDLVDKIIADRKASGEQSDDLLTHMLN
241	GKDPETGEPLDDEN IRYQ I ITFL I AGHETTSGLLSFALYFLVKNPEVLQKA AEEA ARVLV
301	DPVPSYKQVKQLKYVGMVLNEALRLWPTAPAFSLYAKEDTVLGGEYPLERGDELMVL1PQ
361	LHRDKTIWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRACIGQQFALHEATLVLGMMLK
421	HEDFEDHTAYELDI KETUTUKPEGEVVKAKSKKI PLGGI PSPSTEQSAKKVRKKAENAHN
481	TPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVTASYNGH
541	PPDNAKQFVDWEDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFTDETLAAKGAENTAD
601	RGEADASDOFEGTYEEWREHMWSDVAAYFNLDTENSEDNKSTLSLQFVDSAADMPLAKMH
661	GAFSTNVVASKELQQPGSARSTRHLETELPKEASYQEGDHLGVTPRNYEGTVNRVTARFG
721	LDASQQTRLEAEEEKLAHLPLAKTVSVEELLQYVELQDPVTRTQLRAMAAKTVCPPHKVE
781	LEALLEKQAYKEQVLAKRETMLELLEKYPACEMKFSEFIALLPSIRPRYYSISSSPRVDE
841	KQASTTVSVVSGRAWSGYGEYKGTASNYLAELQEGDTTTCFTSTPQSEFTLPKDPETPLT
901	MVGPGTGVAPFRGFVQARKQLKEQGQSLGEAHLYFGCRSPHEDYLYQEELENAQSEGIIT
961	LHTAFSRMPNQPKTYVQHVMEQDGKKLIELLDQGAHFYICGDGSQMAPAVEATLMKSYAD
1021	VHQVSEADARLWLQQLEEKGRYAKDVWAG~

 $[\]divideontimes$ An amino acid sequence of mutants produced by site-directed mutation of wild-type CYP102A1 starts from threonine (T), which is a second amino acid, rather than methionine (M).

[Fig. 2]

5 - ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTA AACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAA TTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGC GATGAATCACGCTTTGATAAAAACTTAAGTCAAGCGCTTAAATTTGTACGTGATTTTGCAGGA GACGGGTTATTTACAAGCTGGACGCATGAAAAAATTGGAAAAAAGCGCATAATATCTTACTT CCAAGCTTCAGTCAGCAGGCAATGAAAGGCTATCATGCGATGATGGTCGATATCGCCGTGCAG CTTGTTCAAAAGTGGGAGCGTCTAAATGCAGATGAGCATATTGAAGTACCGGAAGACATGACA CGTTTAACGCTTGATACAATTGGTCTTTGCGGCTTTAACTATCGCTTTAACAGCTTTTACCGA GATCAGCCTCATCCATTTATTACAAGTATGGTCCGTGCACTGGATGAAGCAATGAACAAGCTG CAGCGAGCAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATC $oldsymbol{A} oldsymbol{\mathsf{A}} oldsymbol{\mathsf{G}} oldsymbol{\mathsf{A}} oldsymbol{\mathsf{C}} oldsymbol{\mathsf{A}} oldsym$ GATGATTTATTAACGCATATGCTAAACGGAAAAGATCCAGAAACGGGTGAGCCGCTTGATGAC GAGAACATTCGCTATCAAATTATTACATTCTTAATTGCGGGACACGAAACAACAAGTGGTCTT TTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACATGTATTACAAAAAGCAGCAGAAGAA GGCATGGTCTTAAACGAAGCGCTGCGCTTATGGCCAACTGCTCCTGCGTTTTCCCTATATGCA AAAGAAGATACGGTGCTTGGAGGAGAATATCCTTTAGAAAAAGGCGACGAACTAATGGTTCTG ATTCCTCAGCTTCACCGTGATAAAACAATTTGGGGAGACGATGTGGAAGAGTTCCGTCCAGAG CGTTTTGAAAATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGT GCGTGTATCGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAA CACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAAACTTTAACGTTAAAA CCTGAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAATTCCGCTTGGCGGTATTCCTTCACCT AGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAACGCTCATAATACGCCGCTG CTTGTGCTATACGGTTCAAATATGGGAACAGCTGAAGGAACGGCGCGTGATTTAGCAGATATT GCAATGAGCAAAGGATTTGCACCGCAGGTCGCAACGCTTGATTCACACGCCGGAAATCTTCCG CGCGAAGGAGCTGTATTAATTGTAACGGCGTCTTATAACGGTCATCCGCCTGATAACGCAAAG CAATTTGTCGACTGGTTAGACCAAGCGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTA TTTGGATGCGGCGATAAAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAA ACGCTTGCCGCTAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGAC ${\tt TTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTAAC}$ CTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGACAGCGCC CTTCAACAGCCAGGCAGTGCACGAAGCACGCGACATCTTGAAATTGAACTTCCAAAAGAAGCT TCTTATCAAGAAGGAGATCATTTAGGTGTTATTCCTCGCAACTATGAAGGAATAGTAAACCGT GTAACAGCAAGGTTCGGCCTAGATGCATCACAGCAAATCCGTCTGGAAGCAGAAGAAGAAAAA TTAGETEATTTGCCACTCGCTAAAACAGTATCCGTAGAAGAGCTTCTGCAATACGTGGAGCTT CAAGATCCTGTTACGCGCACGCAGCTTCGCGCAATGGCTGCTAAAACGGTCTGCCCGCCGCAT AAAGTAGAGCTTGAAGCCTTGCTTGAAAAGCAAGCCTACAAAGAACAAGTGCTGGCAAAACGT TTAACAATGCTTGAACTGCTTGAAAAATACCCGGCGTGTGAAATGAAATTCAGCGAATTTATC GCCCTTCTGCCAAGCATACGCCCGCGCTATTACTCGATTTCTTCATCACCTCGTGTCGATGAA AAACAAGCAAGCATCACGGTCAGCGTTGTCTCAGGAGAAGCGTGGAGCGGATATGGAGAATAT AAAGGAATTGCGTCGAACTATCTTGCCGAGCTGCAAGAAGGAGATACGATTACGTGCTTTATT TCCACACCGCAGTCAGAATTTACGCTGCCAAAAGACCCTGAAACGCCGCTTATCATGGTCGGA CCGGGAACAGGCGTCGCGCGTTTAGAGGCTTTGTGCAGGCGCGCAAACAGCTAAAAGAACAA GGACAGTCACTTGGAGAAGCACATTTATACTTCGGCTGCCGTTCACCTCATGAAGACTATCTG ${ t TATCAAGAAGAGCTTGAAAACGCCCAAAGCGAAGGCATCATTACGCTTCATACCGCTTTTTCT$ CGCATGCCAAATCAGCCGAAAACATACGTTCAGCACGTAATGGAACAAGACGGCAAGAAATTG ATTGAACTTCTTGATCAAGGAGCGCACTTCTATATTTGCGGAGACGGAAGCCAAATGGCACCT CGCTTATGGCTGCAGCAGCTAGAAGAAAAAGGCCGATACGCAAAAGACGTGTGGGCTGGGTAA-3

[Fig. 3] Amino acid sequence of wild-type CYP102A1 mutant #16 (M16)

.1	MET I KEMPQPK TFGELKNLPLLNTDKPVQALMK TADELGE I FKFEAPGRYTRYLSSQRLTK
61	EACDESRFDKNLSQALKFVRDFAGDSILFTSWTHEKNWKKAHNTLLPSFSQQAMKGYHAMM
121	VDTAVQLVQKWERLNADEHTEVPEDMTRLTI,DTTGLCGFNYRFNSFYRDQPHPFTTSMVR
181	ALDEAMNKLQRANPDDPAYDENKRQFQED IKVMNDLVDKT I ADRKASGEQSDDLLTIMLN
241	GKDPETGEPLDDEN I RYQ I I TFL I AGHETTSGLLSFAL Y FL VKNPHVLQKA AEEA ARVL V
301	DPVPSYKQVKQLKYVGNVLNEALRLWPTAPAFSLYAKEDTVLGGEYPLEKGDELVVLIPQ
361	LHRDKTIWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRACIGQQFALHEATLVLGMMLK
421	HPDFEDHTNYELDIKETLTLKPEGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKAENAHN
481	TPLLVLYGSNMGTAEGTARDLADI AMSKGFAPQVATLDSHAGNLPREGAVLI VTASYNGH
541	PPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAFTDETLAAKGAENTAD
601	RGEADASDDFEGTYEEWREHMWSDVAAYFNLDTENSEDNKSTLSLQFVDSAADMPLAKMH
661	GAFSTNVVASKELQQPGSARSTRIILETELPKEASYQEGDIILGVTPRNYEGTVNRVTARFC
721	LDASQQTRLEAEEEKLAHLPLAKTVSVEELLQYVELQDPVTRTQLRAMAAKTVCPPHKVE
781	LEALLEKQAYKEQVLAKRLTMLELLEKYPACEMKFSEFTALLPSTRPRYYSTSSSPRVDE
841	KQASITVSVVSGEAWSGYGEYKGIASNYLAELQEGDTITCFISTPQSEFTLPKDPETPLI
901	MVGPGTGVAPFRGFVQARKQLKEQGQSLGEAHLYFGCRSPHEDYLYQEELENAQSEGIIT
961	LHTAFSRMPNQPKTYVQHVMEQDXJKKLTELLDQGAHFYTCGDGSQMAPAVEATLMKSYAD
1021	VHQVSEADARL#LQQLEEKGRYAKDVWAG~

[Fig. 4]

5' -ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTA AACACAGATAAACGGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAA TTCGAGGCGCCTGGTCTTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGC GATGAATCACGCTTTGATAAAAACTTAAGTCAAGCGCTTAAATTTGTACGTGATATTGCAGGA GACGGGTTAGTTACAAGCTGGACGCATGAAAAAATTGGAAAAAAGCGCATAATATCTTACTT CCAAGCTTCAGTCAGCAGGCAATGAAAGGCTATCATGCGATGATGGTCGATATCGCCGTGCAG CTTGTTCAAAAGTGGGAGCGTCTAAATGCAGATGAGCATATTGAAGTACCGGGAGACATGACA CGTTTAACGCTTGATACAATTGGTCTTTGCGGCTTTAACTATCGCTTTAACAGCTTTTACCGA GATCAGCCTCATCCATTTATTACAAGTATGGTCCGTGCACTGGATGAAGCAATGAACAAGCAG CAGCGAGCAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTICAAGAAGATATC GATGATTTATTAACGCATATGCTAAACGGAAAAGATCCAGAAACGGGTGAGCCGCTTGATGAC GAGAACATTCGCTATCAAATTATTACATTCTTAATTGCGGGACACGTAACAACAAGTGGTCTT TTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACATGTATTACAAAAAGCAGCAGAAGAA GGCATGGTCTTAAACGAAGCGCTGCGCTTATGGCCAACTGCTCCTGCGTTTTCCCTATATGCA AAAGAAGATACGGTGCTTGGAGGAGAATATCCTTTAGAAAAAGGCGACGAACTAATGGTTCTG ATTCCTCAGCTTCACCGTGATAAAACAATTTGGGGAGACGATGTGGAAGAGTTCCGTCCAGAG CGTTTTGAAAATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGT GCGTGTATCGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAA CACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAAACTTTAACGTTAAAA CCTGAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAATTCCGCTTTGGCGGTATTCCTTCACCT AGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAACGCTCATAATACGCCGCTG CTTGTGCTATACGGTTCAAATATGGGAACAGCTGAAGGAACGGCGCGTGATTTAGCAGATATT GCAATGAGCAAAGGATTTGCACCGCAGGTCGCAACGCTTGATTCACACGCCGGAAATCTTCCG CGCGAAGGAGCTGTATTAATTGTAACGGCGTCTTATAACGGTCATCCGCCTGATAACGCAAAG CANTTIGICGACTOGITAGACCAAGCGICIGCTGATGAAGTAAAAGGCGITCGCTACTCCGIA TTTGGATGCGGCGATAAAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAA ACGCTTGCCGCTAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGAC TTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTAAC $\mathtt{CTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGACAGCGCC$ CTTCAACAGCCAGGCAGTGCACGAAGCACGCGACATCTTGAAATTGAACTTCCAAAAGAAGCT TCTTATCAAGAAGGAGATCATTTAGGTGTTATTCCTCGCAACTATGAAGGAATAGTAAACCGT GTAACAGCAAGGTTCGGCCTAGATGCATCACAGCAAATCCGTCTGGAAGCAGAAGAAGAAGAAAA TTAGCTCATTTGCCACTCGCTAAAACAGTATCCGTAGAAGAGCTTCTGCAATACGTGGAGCTT CAAGATCCTGTTACGCGCACGCAGCTTCGCGCAATGGCTGCTAAAACGGTCTGCCCGCCGCAT AAAGTAGAGCTTGAAGCCTTGCTTGAAAAGCAAGCCTACAAAGAACAAGTGCTGGCAAAACGT TTAACAATGCTTGAACTGCTTGAAAAATACCCGGCGTGTGAAATGAAATTCAGCGAATTTATC GCCCTTCTGCCAA6CATACGCCCGCGCTATTACTCGATTTCTTCATCACCTCGTGTCGATGAA AAACAAGCAAGCATCACGGTCAGCGTTGTCTCAGGAGAAGCGTGGAGCGGATATGGAGAATATAAAGGAATIGCGTCGAACTATCTTGCCGAGCTGCAAGAAGGAGATACGATTACGTGCTTTATT TCCACACCGCAGTCAGAATTTACGCTGCCAAAAGACCCTGAAACGCCGCTTATCATGGTCGGA ${\tt CCGGGAACAGGCGTCGCGCGTTTAGAGGCTTTGTGCAGGCGCGCAAACAGCTAAAAGAACAA$ GGACAGTCACTTGGAGAAGCACATTTATACTTCGGCTGCCGTTCACCTCATGAAGACTATCTG TATCAAGAAGAGCTTGAAAACGCCCAAAGCGAAGGCATCATTACGCTTCATACCGCTTTTTCT CGCATGCCAAATCAGCCGAAAACATACGTTCAGCACGTAATGGAACAAGACGGCAAGAAATTG ATTGAACTTCTTGATCAAGGAGCGCACTTCTATATTTGCGGAGACGGAAGCCAAATGGCACCT CGCTTATGGCTGCAGCAGCTAGAAGAAAAAGGCCGATACGCAAAAGACGTGTGGGCTGGGTAA-3'

[Fig. 5] Amino acid sequence of wild-type CYP102A1 mutant #17 (M17)

1	MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGLVTRYLSSQRLIK
	BELLIKERI KETELLED IN TERLEBERT DESTENDE I VERLESTE DE LE PERFERDE DE LE PERFERDITE.
61	EACDGSRFDKNLSQALKFVRDIAGDGLVTSWTHEKNWKKAHNILLPSFSQQAMKGYHAMM
121	VDIAVQLVQKWERLNADEHIEVPGEMTRETEDTIGE.CGFNYRFNSFYRDQPHPFITSMVR
181	ALDEAMNKQQRANPODPAYDENKRQFQEDIKVMNDLVDKIIADRKASGEQSDDLLTHMLN
241	GKDPETGEPLDDEN IRYQI I TFL I AGHVTTSGLLSFALYFL VKNPHVLQKAAEEAARVL V
301	DPVPSYKQVKQLKYVGNVLNEALRIWPTAPAFSLYAKEDTVLGGEYPLEKGDELMVLIPQ
361	LHRDKTIWGDDVEEFRPERFENPSAIPQHAFKPFGNGQRACIGQQFALHEATLVLGMMLK
421	HFDFEDHINYELDIKETLILKPEGFVVKAKSKKIPLGG IPSPSTEQSAKKVRKKVENAHN
481	TPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSHAGNLPREGAVLIVTASYNGH
54.1	PPDNAKQFVDWLDQASADDVKGVRYSVFGCGDKNWATTYQKVPAF I DETLAAKGAEN I AD
601	RGEADASDDFEGTYEEWREHMWSDVAAYFNLD1ENSEDNKSTLSLQFVDSAADMPLAKMH
661	GAFSANVVASKELQQLGSERSTRHLETALPKEASYQEGDHLGVTPRNYEGTVNRVTARFG
721	LDASQQTRLEAEEEKLAHLPLGKTVSVEELLQYVELQDPVTRTQLRAMAAKTVCPPHKVE
781	LEALLEKQAYKEQVLAKRUTMLELLEKYPACEMEFSEFTALLPSTSPRYYSTSSSPHVDE
841	KQASITVSVVSGEAWSGYGEYKGIASNYLANLQEGDTITCFVSTPQSGFTLPKDSETPLI
901	MVGPGTGVAPFRGFVQARKQLKEQGQSLGEAHLYFGCRSPHEDYLYQBBLENAQNEG11T
961	LHTAFSRVPNQPKTYVQHVMERDGKKLIELLDQGAHFYICGDGSQMAPDVEATLMKSYAD
1021	VYEVSEADARLWLQQLEEKGRYAKDVWAG~

[Fig. 6]

5 ~ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTA <u>AACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAA</u> TTCGAGGCGCCTGGTCTTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGC GATGGATCACGCTTTGATAAAAACTTAAGTCAAGCGCTTAAATTTGTACGTGATATTGCAGGA GACGGGTTAGTTACAAGCTGGACGCATGAAAAAATTGGAAAAAAGCGCATAATATCTTACTT CCAAGCITCAGTCAGCAGGCAATGAAAGGCTATCATGCGATGATGGTCGATATCGCCGTGCAG CTTGTTCAAAAGTGGGAGCGTCTAAATGCAGATGAGCATATTGAAGTACCGGGAGACATGACA CGTTTAACGCTTGATACAATTGGTCTTTGCGGCTTTAACTATCGCTTTAACAGCTTTTACCGA GATCAGCCTCATCCATTTATTACAAGTATGGTCCGTGCACTGGATGAAGCAATGAACAAGCAG CAGCGAGCAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATC A A GGTG A TG A A CGA CCTA GTA GA TA A A A TTA TTG CA GA TCG CAA A GCA A GCGGTGA A CAA A GC GATGATTTATTAACGCATATGCTAAACGGAAAAGATCCAGAAACGGGTGAGCCGCTTGATGAC GAGAACATTCGCTATCAAATTATTACATTCTTAATTGCGGGACACGTAACAACAAGTGGTCTT TTATCATITGCGCTGTATTTCTTAGTGAAAAATCCACATGTATTACAAAAAGCAGCAGAAGAA GGCATGGTCTTAAACGAAGCGCTGCGCTTATGGCCAACTGCTCCTGCGTTTTCCCTATATGCA A A A G A A G A T A C G G T G C T T G G A G G A G A A T A T C C T T T A G A A A A G G C G A C G A A C T A A T G G T T C T G ATTOCTCAGCITCACCGTGATAAAACAATTTGGGGAGACGATGTGGAAGAGTTCCGTCCAGAG CGTTTTGAAAATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGT GCGTGTATCGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAA CACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAAACTTTAACGTTAAAA CCTGAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGTATTCCTTCACCT AGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAACGCTCATAATACGCCGCTG CTTGTGCTATACGGTTCAAATATGGGAACAGCTGAAGGAACGGCGCGTGATTTAGCAGATATT GCAATGAGCAAAGGATTTGCACCGCAGGTCGCAACGCTTGATTCACACGCCGGAAATCTTCCG CGCGAAGGAGCTGTATTAATTGTAACGGCGTCTTATAACGGTCATCCGCCTGATAACGCAAAG CAATTTGTCGACTGGTTAGACCAAGCGTCTGCTGATGAAGTAAAAGGCGTTCGCTACTCCGTA TTTGGATGCGGCGATAAAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAA ACGCTTGCCGCTAAAGGGGCAGAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGAC ${ t TTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTAAC$ CTCGACATTGAAAACAGTGAAGATAATAAATCTACTCITTCACTTCAATTTGTCGACAGCGCC CTTCAACAGCCAGGCAGTGCACGAAGCACGCGACATCTTGAAATTGAACTTCCAAAAGAAGCT TCTTATCAAGAAGGAGATCATTTAGGTGTTATTCCTCGCAACTATGAAGGAATAGTAAACCGT GTAACAGCAAGGTTCGGCCTAGATGCATCACAGCAAATCCGTCTGGAAGCAGAAGAAGAAAAA TTAGCTCATTTGCCACTCGCTAAAACAGTATCCGTAGAAGAGCTTCTGCAATACGTGGAGCTT CAAGATCCIGITACGCGCACGCAGCTTCGCGCAATGGCTGCIAAAACGGTCTGCCCGCCGCAT AAAGTAGAGCTTGAAGCCTTGCTTGAAAAGCAAGCCTACAAAGAACAAGTGCTGGCAAAACGT TTAACASTGCTTGAACTGCTTGAAAAATACCCGGCGTGTGASATGAAATTCAGCGAATTTATC GCCCTTCTGCCAAGCATACGCCCGCGCTATTACTCGATTTCTTCATCACCTCGTGTCGATGAA AAACAAGCAAGCATCACGGTCAGCGTTGTCTCAGGAGAGCGTGGAGCGGATATGGAGAATAT AAAGGAATTGCGTCGAACTATCTTGCCGAGCTGCAAGAAGGAGATACGATTACGTGCTTTATT TCCACACCGCAGTCAGAATTTACGCTGCCAAAAGACCCTGAAACGCCGCTTATCATGGTCGGA CCGGGAACAGGCGTCGCGCCGTTTAGAGGCTTTGTGCAGGCGCGCAAACAGCTAAAAGAACAA GGACAGTCMCTTGGAGAAGCACATTTATACTTCGGCTGCCGTTCACCTCATGAAGACTATCTG TATCAAGAAGAAGCTTGAAAACGCCCAAAGCGAAGGCATCATTACGCTTCATACCGCTTTTTCT CCCATGCCAAATCAGCCGAAAACATACGTTCAGCACGTAATGGAACAAGACGCCAAGAAATTG ATTGAACTTCTIGATCAAGGAGCGCACTTCTATATTTGCGGAGACGGAAGCCAAATGGCACCT CGCTTATGGCTGCAGCAGCTAGAAGAAAAAGGCCGATACGCAAAAGACGTGTGGGCTGGGTAA-3

[Fig. 7]

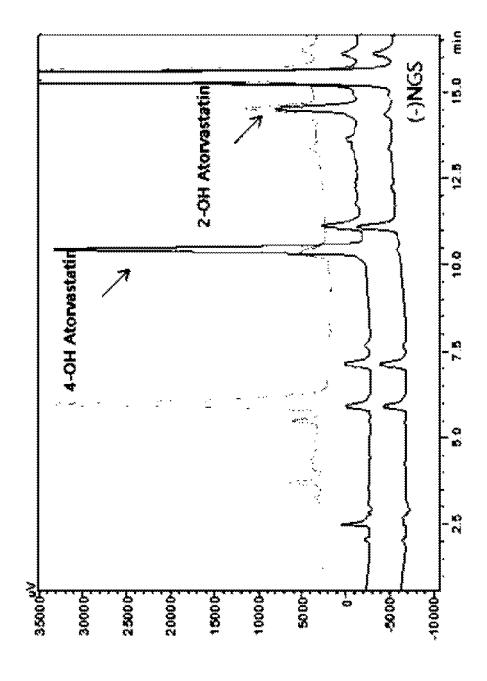
Amino acid sequence of chimera M16A1V2 derived from wild-type CYP102A1 mutant #16 (M16)

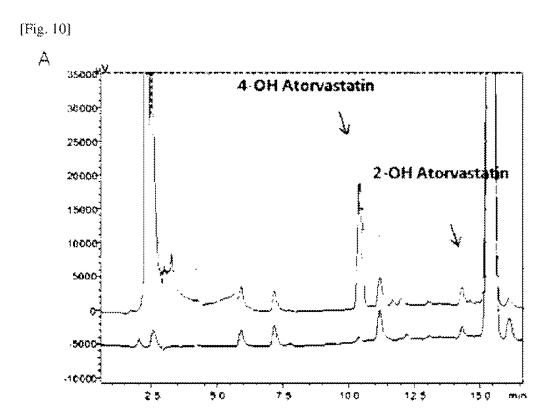
1	MT1KEMPQPKTFGELKNLPLLNTDKPVQALMK1ADELGE1FKFEAPGRVTRYLSSQRL1K
61	EACDESRFDKNLSQALKFYRDFAGDGLFTSWTHEKNWKKAHNTLLPSFSQQAMKGYHAMM
121	VD1AVQLVQXWERLNADEH1EVPEDMTRLTLDT1GLCGFNYRFNSFYRDQPHPF1TSMVR
181	ALDEAMNKLQRANPDDPAYDENKRQFQEDTKVMNDLVDKTTADRKASGEQSDDLLTHMLN
241	GKDPETGEPLDDEN I RYQ I I TFL I AGHETTSGLLSFALYFLVKNPHVLQKAAEEAARVLV
301	DPVPSYKQVKQLKYVGMVLNEALRLWPTAPAFSLYAKEDTVLGGEYPLEXGDELMVLTPQ
361	LHRDKT1WGDDVEEFRPERFENPSA1PQHAFKPFGNGQRAC1GQQFALHEATLVLGMMLK
421	HFDFEDHTNYELDIKETI.TLKPEGFVVKAKSRKIPLGGIPSPSTEQSAKKVRKKVEXAHN
481	TPLLVLYGSNMGTAFÆTARÐLAÐFAMSKGFAPQVATLÐSHAGNLPREGAVLFVTASYNGH
541	PPDNAKQFVDWLDQASADDVKGVRYSVFGCGDKNWATTYQKVPAFIDETLAAKGAENIAD
601	RGEADASDDFEGTYEEWREIMWSDVAAYFNLDTEASEDNKSTLSLQFVDSAADMPLAKMH
661	GAFSANVVASKELQQLGSERSTRHLETALPREASYQEGDHLGVTPRNYEGTVNRVTARFG
721	LDASQQIRLEAEEEKLAHLPLGKTVSVEELLQYVELQDPVTRTQLRAMAAKTVCPPHKVE
781	LEALLEKQAYKEQVLAKRLTMLELLEKYPACEMEFSEFTALLPSISPRYYSISSSPHVDE
841	KQASTTYSVVSGEAWSGYGEYKGTASNYLANLQEGDTTTCFVSTPQSGFTLPKDSETPLT
901	MVGPGTGVAPFRGFVQARKQLKEQGQSLGEAHLYFGCRSPHEDYLYQEELENAQNEG I IT
961	LHTAFSRVPNQPKTYVQHVMERDGKKLIELLDXGA8FYICGDGSQMAPDVEATDNKSYAD
1021	VYEVSEADAREWLQQLEEKGRYAKDVWAG-

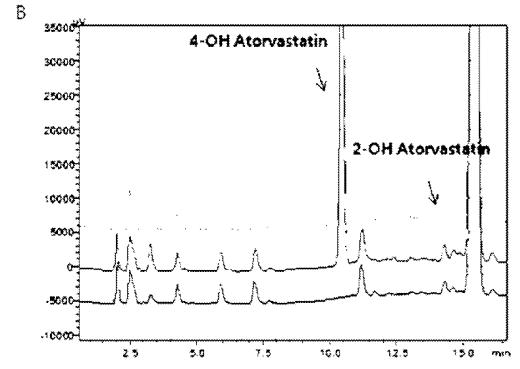
[Fig. 8]

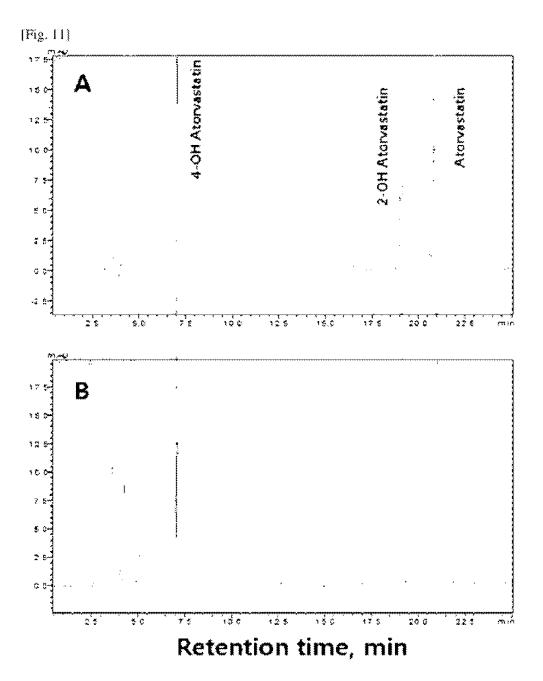
5° -ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTA AACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAA TTCGAGGCGCCTGGTCTTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGC GATGAATCACGCTTTGATAAAAACTTAAGTCAAGCGCTTAAATTTGTACGTGATATTGCAGGA GACGGGTTAGTTACAAGCTGGACGCATGAAAAAATTGGAAAAAAGCGCATAATATCTTACTT CCAAGETTCAGTCAGCAGGCAATGAAAGGCTATCATGCGATGATGGTCGATATCGCCGTGCAG CTTGTTCAAAAGTGGGAGCGTCTAAATGCAGATGAGCATATTGAAGTACCGGGAGACATGACA **CGTTTAACGCTTGATACAATTGGTCTTTGCGGCTTTAACTATCGCTTTAACAGCTTTTACCGA** GATEAGCCTEATCCATTTATTACAAGTATGGTCCGTGCACTGGATGAAGCAATGAACAAGCAG CAGCGAGCAAATCCAGACGACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATC GATGATTTATTAACGCATATGCTAAACGGAAAAGATCCAGAAACGGGTGAGCCGCTTGATGAC GAGAACATTCGCTATCAAATTATTACATTCTTAATTGCGGGACACGTAACAACAAGTGGTCTT TTATCATTTGCGCTGTATTTCTTAGTGAAAAATCCACATGTATTACAAAAAGCAGCAGAAGAA GGCATGGTCTTAAACGAAGCGCTGCGCTTATGGCCAACTGCTCCTGCGTTTTCCCTATATGCA AAAGAAGATACGGTGCTTGGAGGAGAATATCCTTTAGAAAAAGGCGACGAACTAATGGTTCTG ATTCCTCAGCTTCACCGTGATAAAACAATTTGGGGAAGACGATGTGGAAGAGTTCCGTCCAGAG CGTTTTGAAAATCCAAGTGCGATTCCGCAGCATGCGTTTAAACCGTTTGGAAACGGTCAGCGT GCGTGTATCGGTCAGCAGTTCGCTCTTCATGAAGCAACGCTGGTACTTGGTATGATGCTAAAA CACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATATTAAAGAAACTTTAACGTTAAAA CCTGAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCGCTTGGCGGTATTCCTTCACCT AGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGTAGAAAACGCTCATAATACGCCGCTG CTTGTGCTATACGGTTCAAATATGGGAACAGCTGAAGGAACGGCGCGTGATTTAGCAGATATT GCANTGAGCNANGGATTTGCACCGCAGGTCGCANCGCTTGATTCACACGCCGGANATCTTCCG CGCGAAGGAGCTGTATTAATTGTAACGGCGTCTTATAACGGTCATCCGCCTGATAACGCAAAG CAATTTGTCGACTGGTTAGACCAAGCGTCTGCTGATGATGTAAAAGGCGTTCGCTACTCCGTA TTTGGATGCGGCGATAAAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTTATCGATGAA ACGCTTGCCGCTAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAGCGACGAC TTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTAAC CTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGACAGCGCC CTTCAACAGCTAGGCAGTGAACGAAGCACGCGACATCTTGAAATTGCACTTCCAAAAGAAGCT **TOTTATCAAGAAGGAGATCATTTAGGTGTTATTCCTCGCAACTATGAAGGAATAGTAAACCGT** GTAACAGCAAGGTTCGGCCTAGATGCATCACAGCAAATCCGTCTGGAAGCAGAAGAAGAAAAA TTAGCTCATTTGCCACTCGGTAAAACAGTATCCGTAGAAGAGCTTCTGCAATACGTGGAGCTT CAAGATCCTGTTACGCGCACGCAGCTTCGCGCAATGGCTGCTAAAACGGTCTGCCCGCCGCAT AAAGTAGAGCTTGAAGCCTTGCTTGAAAAGCAAGCCTACAAAGAACAAGTGCTGGCAAAACGT TTAACAATGCTTGAACTGCTTGAAAAATACCCGGCGTGTGAAATGGAATTCAGCGAATTTATC GCCCTTCTGCCAAGCATAAGCCCGCGCTATTACTCGATTTCTTCATCACCTCATGTCGATGAA AAACAAGCAAGCATCACGGTCAGCGTTGTCTCAGGAGAAGCGTGGAGCGGATATGGAGAATAT AAAGGAATTGCGTCGAACTATCTTGCCGATCTGCAAGAAGGAGATACGATTACGTGCTTTGTT TCCACACCGCAGTCAGGATTTACGCTGCCAAAAGACTCTGAAACGCCGCTTATCATGGTCGGA CCGGGAACAGGCGTCGCGCCGTTTAGAGGCTTTGTGCAGGCGCGCAAACAGCTAAAAGAACAA GGACAGTCACTTGGAGAAGCACATTTATACTTCGGCTGCCGTTCACCTCATGAAGACTATCTG TATCAAGAAGAGCTTGAAAACGCCCAAAACGAAGGCATCATTACGCTTCATACCGCTTTTTCT CGCGTGCCAAATCAGCCGAAAACATACGTTCAGCACGTAATGGAACGAGACGGCAAGAAATTG ATTGAACTTCTTGATCAAGGAGCGCACTTCTATATTTGCGGAGACGGAAGCCAAATGGCACCT CGCTTATGGCTGCAGCAGCTAGAAGAAAAAGGCCGATACGCAAAAGACGTGTGGGCTGGGTAA~3'

[Fig. 9]









0 50

0 25

450

500

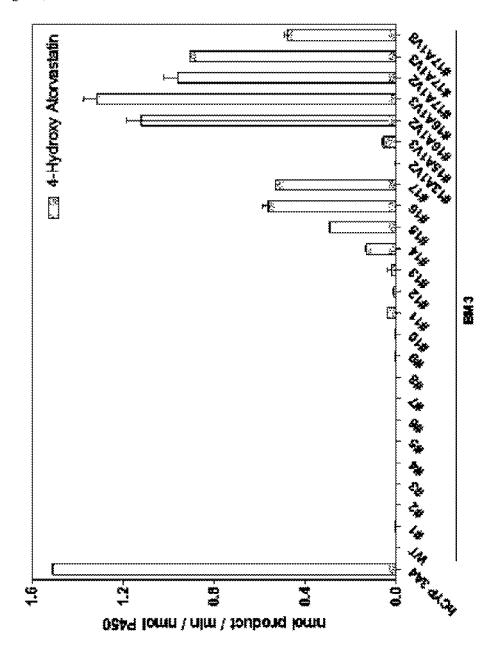
550

800

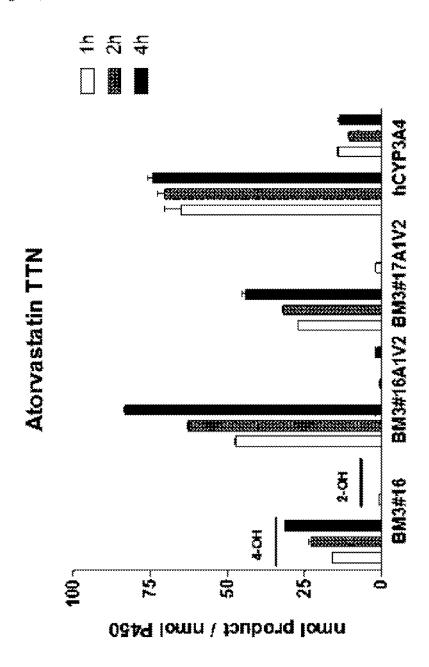
850

[Fig. 12] 573 7.5~ 50 2.5 و ۾ ۾ 550 500 23 B 1 5 10 e é o 850 inten an 000,000; 0.75

[Fig. 13]



[Fig. 14]



NOVEL METHOD FOR PREPARING METABOLITES OF ATORVASTATIN USING BACTERIAL CYTOCHROME P450 AND COMPOSITION THEREFOR

TECHNICAL FIELD

[0001] The present invention relates to a novel method for preparing metabolites of atorvastatin using bacterial cytochrome P450 and a composition therefor.

BACKGROUND ART

[0002] Atorvastatin is well known as an anti-hyperlipidemic agent, an antihypercholesterolemic agent, or a cholesterol-lowering agent. Oxidative metabolism of atorvastatin in human liver is mediated by mainly cytochrome P450 3A (CYP3A) enzymes, particularly, cytochrome P450 3A4 (CYP3A4), and the following two metabolites, that is, orthohydroxy atorvastatin (ortho-OH atorvastatin or 2-OH atorvastatin) and parahydroxy atorvastatin (para-OH atorvastatin or 4-OH atorvastatin) are generated.

[0003] After oral ingestion, atorvastatin, which is an inactive lactone, is hydrolyzed to the corresponding β -hydroxy acid form. This is a main metabolite and an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol.

[0004] In addition to the P450-mediated oxidation and β-oxidation processes, glucuronidation constitutes a common metabolic pathway for statins (Prueksaritanont et al., Drug Metab. Dispos. 30:505-512, 2002). The metabolites resulting from microsomal oxidation of atorvastatin by P450 enzymes are effective inhibitors of HMG-CoA reductase. In addition, it has been suggested that the metabolites may contribute to the cholesterol-lowering effect of atorvastatin.

[0005] Cytochrome P450 enzymes (P450s or CYPs) are large families consisting of enzymes serving as remarkably diverse oxygenation catalysts in throughout nature from archaea, bacteria, fungi, plants, and animals up to humans (http://drnelson.uthsc.edu/CytochromeP450.html). Due to

the catalytic diversity and broad substrate range of P450s, they are attractive biocatalyst candidates for the production of fine chemicals, including pharmaceuticals.

[0006] However, in spite of the potential use of mammalian P450s in various biotechnology fields, they are not suitable as biocatalysts because of their low stability, low catalytic activity, and low affordability.

[0007] In the case in which a pro-drug is converted into a biologically "active metabolite" by human hepatic P450s during drug development, a large amount of pure metabolites are required in order to research into effect, toxicity, pharmacokinetics of the drug, or the like. Further, in the case in which the metabolite itself has biological activity, it may be advantageous to directly administer the metabolite to the body. Therefore, it is important to prepare the metabolite on a large scale

[0008] However, since there are various problems in chemically synthesizing pure metabolites, P450 may be used in order to prepare the metabolites of a drug or drug candidates as an alternative for chemical synthesis of the metabolites. The metabolite preparation has been reported using human P450s expressed in *Escherichia coli* (Yun et al., Curr. Drug Metab. 7:411-429, 2006) and in insect cells (Rushmore et al., Metab. Eng. 2:115-125, 2000; Vail et al., J. Ind. Microbiol. Biotechnol. 32:67-74. 2005).

[0009] However, since these systems are still costly and have low productivities due to limited stabilities and slow reaction rates, a method of using engineered bacterial P450 enzymes having the desired catalyst activity has been suggested as an alternative for producing human metabolite.

[0010] Meanwhile, P450 BM3 (CYP102A1) from *Bacillus megaterium* has strong similarity to eukaryotic members of the CYP4A (fatty acid hydroxylase) family. It has been reported that CYP102A1 mutants oxidizes several human P450 substrates to produce the metabolite with higher activity (Kim et al., Protein Expr. Purif. 57:188-200, 2008a). Further, CYP102A1 is a versatile monooxygenase capable of working on various substrates (Di Nardo et al., J. Biol. Inorg. Chem. 12:313-323, 2007).

[0011] Recently, it has been reported that CYP102A1 mutants may produce larger quantities of the human metabolites of drugs, which may be difficult to be synthesized (Otey et al., Biotechnol. Bioeng. 93:494-499, 2005). Therefore, as an alternative method of preparing the metabolites, it may be considered to use CYP102A1 engineered so as to have the desired properties.

[0012] Several amino acid residues in CYP102A1 were mutated to generate mutant enzymes having increased activity toward human P450 substrates by the present inventors (Yun et al., Trends Biotechnol. 25:289-298, 2007 and other references cited in the article), and it was confirmed that specific mutants among these mutant enzymes may enable the CYP102A1 enzyme to catalyze O-deethylation and 3-hydroxylation of 7-ethoxycoumarin (Kim et al. Drug Metab. Dispos. 36:2166-2170, 2008a).

[0013] Therefore, while conducting research for directly using the atorvastatin metabolites as a drug, the present inventors discovered bacterial enzymes capable of oxidizing atorvastatin, which is known as a human P450 substrate, to produce 2-hydroxylated product and 4-hydroxylated product, which are human metabolites, and a biological preparation method using the same, thereby completing the present invention.

DISCLOSURE OF INVENTION

Technical Problem

[0014] An object of the present invention is to provide a bacterial enzyme capable of oxidizing atorvastatin to preparing 4-hydroxylated product or 2-hydroxylated product, which are human metabolites, on a large scale.

[0015] In addition, another object of the present invention is to provide a composition for preparing 2-hydroxylated product or 4-hydroxylated product from atorvastatin containing the enzyme.

[0016] Further, another object of the present invention is to provide a method for preparing 2-hydroxylated product or 4-hydroxylated product from atorvastatin including reacting the enzyme with atorvastatin.

[0017] Furthermore, another object of the present invention is to provide a kit for preparing 2-hydroxylated product or 4-hydroxylated product from atorvastatin containing the enzyme and a reduced nicotinamide adenine dinucleotide phosphate (NADPH)-generating system.

Solution to Problem

[0018] In one general aspect, there is provided a preparation method capable of selectively preparing human metabolites, particularly 2-hydroxylated product or 4-hydroxylated product from atorvastatin on a large scale using wild-type CYP102A1, CYP102A1 mutants, or chimeras derived from CYP102A1 mutants as a bacterial P450 enzyme, and a composition and a kit therefor.

[0019] In the present invention, "the CYP102A1 mutants" have an amino acid sequence of the wild-type CYP102A1 modified by natural or artificial substitution, deletion, addition, and/or insertion. Preferably, amino acid of the CYP102A1 mutant may be substituted with an amino acid that has similar properties as classified below. For example, alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, and tryptophan are classified as nonpolar amino acids and have similar properties to each other. Glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine are neutral amino acids, aspartic acid and glutamic acid are acidic amino acids, and lysine, arginine, and histidine are basic amino acids.

[0020] The CYP102A1 mutants according to the present invention include polypeptide having an amino acid sequence similar to an amino acid sequence of CYP102A1 at an identity level of 50% or more, preferably, 75% or more, and more preferably, 90% or more.

[0021] In the present invention, the terms "chimeric" is used in the case in which at least two binding domains that are different from each other are contained therein. The two binding domains may be derived from different wild-type proteins. The two domains may be derived from the same wild-type protein, but in chimeric protein according to the present invention, the two domains may be positioned in a different arrangement from the corresponding the wild-type CYP102A1 mutant protein by fusing a heme domain of the wild-type CYP102A1 and a reductase domain of natural variants of the wild-type CYP102A1 to each other.

[0022] Hereinafter, the present invention will be described in detail.

[0023] The wild-type CYP102A1, the CYP102A1 mutant, or the chimera derived from the CYP102A1 mutant may be

used as a catalyst in oxidation reaction using atorvastatin that is known as a human P450 substrate as the substrate.

[0024] More specifically, the present inventors clarified that the wild-type CYP102A1, the CYP102A1 mutant, or the chimera derived from the CYP102A1 mutant may be used as a catalyst in oxidation reaction using atorvastatin that is known as a human P450 substrate as the substrate. Particularly, in the case in which human CYP3A4 is used as the catalyst, as the produced atorvastatin metabolites, 2-hydroxylated product and 4-hydroxylated product may not be selectively produced. On the other hand, in the case in which the wild-type CYP102A1 mutant and the chimeras derived from the CYP102A1 according to the present invention are used as the catalyst, large amounts of 2-hydroxylated product and 4-hydroxylated product may be selectively and stably produced.

[0025] The present inventors prepared chimeras (#16A1V2, #17A1V2) derived from the CYP102A1 by selecting several mutants (wild-type CYP102A1 mutants #16 and #17 shown in Tables 2 and 3) with high catalytic activity for some substrates in a human among mutants prepared by over-expressing bacterial wild-type CYP102A1 and site-directed mutants thereof in *E. coli* (See Table 1) and fusing heme domains thereof and reductase domains of natural variants of the wild-type CYP102A1 to each other.

[0026] In the case in which the bacterial wild-type CYP102A1, the prepared mutants thereof (wild-type CYP102A1 mutants #16 and #17 shown in Tables 2 and 3), and chimeras (#16A1V2, #17A1V2) derived from the CYP102A1 was over-expressed in *E. coli* to be reacted with atorvastatin and a NADPH-generating system, it was confirmed that atorvastatin is converted into metabolites in humans through high-performance liquid chromatography (HPLC) (See FIG. 9) and a liquid chromatography-mass spectrometry (LC-MS) spectrum (See FIGS. 11 and 12).

[0027] In the case in which human CYP3A4 is used as the catalyst, as the produced atorvastatin metabolites, 2-hydroxylated product and 4-hydroxylated product may not be selectively produced. On the other hand, it might be appreciated that in the case in which the wild-type CYP102A1 mutant and the chimeras derived from the CYP102A1 according to the present invention are used as the catalyst, 2-hydroxylated product and 4-hydroxylated product may be selectively prepared on a large scale.

[0028] In addition, it might be appreciated that three kinds of mutants (#15, #16, and #17 in Table 2) and five kinds of chimeras (#16A1V2, #16A1V3, #17A1V2, #17A1V3, and #17A1V8) derived from the mutants have a large turnover number among the wild-type CYP102A1 mutants and the chimeras derived from the wild-type CYP102A1 mutants in producing the metabolites of atorvastatin. Particularly, it might be appreciated that the chimera #16A1V2 derived from the CYP102A1 mutant #16 and the chimera #17A1V2 derived from the CYP102A1 mutant #17 have the most excellent turnover number. See FIG. 14.

[0029] Based on the experiment results as described above, the present invention provides a composition for preparing 2-hydroxylated product or 4-hydroxylated product from atorvastatin including at least one enzyme selected from a group consisting of the wild-type CYP102A1, the CYP102A1 mutants, and chimeras derived from the CYP102A1 mutants, [0030] wherein the CYP102A1 mutant has an amino acid sequence changed from that of the wild-type CYP102A1 by at least one substitution selected from a group consisting of

substituting arginine (R) at the amino acid position 47 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting tyrosine (Y) at the amino acid position 51 with an amino acid selected from a group consisting of alanine, valine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting glutamic acid (E) at the amino acid position 64 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, substituting alanine (A) at the amino acid position 74 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, substituting phenylalanine (F) at the amino acid position 81 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, and tryptophan, substituting leucine (L) at the amino acid position 86 with an amino acid selected from a group consisting of alanine, valine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting phenylalanine (F) at amino acid position 87 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, and tryptophan, substituting glutamic acid (E) at the amino acid position 143 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, substituting leucine (L) at the amino acid position 188 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, and substituting glutamic acid (E) at the amino acid position 267 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, and tryptophan, and

[0031] the chimera derived from the CYP102A1 mutant has an amino acid sequence changed from that of the reductase domain of the CYP102A1 mutant by at least one substitution selected from a group of substituting lysine (K) at the amino acid position 474 with threonine (T), substituting alanine (A) at the amino acid position 475 with valine (V), substituting glutamine (Q) at the amino acid position 513 with arginine (R), substituting arginine (R) at the amino acid position 526 with proline (P), substituting glutamine (Q) at the amino acid position 547 with glutamic acid (E), substituting glutamic acid (E) at the amino acid position 559 with aspartic acid (D), substituting leucine (L) at the amino acid position 590 with phenylalanine (F), substituting alanine (A) at the amino acid position 591 with serine (S), substituting aspartic acid (D) at the amino acid position 600 with glutamic acid (E), substituting valine (V) at the amino acid position 625 with leucine (L), substituting aspartic acid (D) at the amino acid position 632 with asparagine (N), substituting aspartic acid (D) at the amino acid position 638 with glutamic acid (E), substituting lysine (K) at the amino acid position 640 with alanine (A), substituting alanine (A) at the amino acid position 652 with serine (S), substituting glycine (G) at the amino acid position 661 with arginine (R), substituting threonine (T) at the amino acid position 665 with alanine (A), substituting glutamine (Q) at the amino acid position 675 with lysine (K), substituting proline (P) at the amino acid position 676 with leucine (L), substituting alanine (A) at the amino acid position 679 with glutamic acid, substituting glutamic acid (E) at the amino acid position 688 with alanine (A), substituting threonine (T) at the amino acid position 716 with alanine (A), substituting alanine (A) at the amino acid position 717 with threonine (T), substituting alanine (A) at the amino acid position 742 with glycine (G), substituting alanine (A) at the amino acid position 783 with valine (V), substituting alanine (A) at the amino acid position 796 with threonine (T), substituting lysine (K) at the amino acid position 814 with glutamic acid (E), substituting isoleucine (I) at the amino acid position 825 with methionine (M), substituting arginine (R) at the amino acid position 826 with serine (S), substituting arginine (R) at the amino acid position 837 with histidine (H), substituting glutamic acid (E) at the amino acid position 871 with asparagine (N), substituting isoleucine (I) at the amino acid position 882 with valine (V), substituting glutamic acid (E) at the amino acid position 888 with glycine (G), substituting aspartic acid (D) at the amino acid position 894 with glycine (G), substituting proline (P) at the amino acid position 895 with serine (S), substituting glycine (G) at the amino acid position 913 with serine (S), substituting glutamic acid (E) at the amino acid position 948 with lysine (K), substituting serine (S) at the amino acid position 955 with asparagine (N), substituting methionine (M) at the amino acid position 968 with valine (V), substituting glutamine (Q) at the amino acid position 971 with glutamic acid (E), substituting methionine (M) at the amino acid position 980 with valine (V), substituting glutamine (Q) at the amino acid position 982 with arginine (R), substituting alanine (A) at the amino acid position 1009 with aspartic acid (D), substituting aspartic acid (D) at the amino acid position 1020 with glutamic acid (E), substituting histidine (H) at the amino acid position 1022 with tyrosine (Y), substituting glutamine (Q) at the amino acid position 1023 with lysine (K) and glutamic acid (E), and substituting glycine (G) at the amino acid position 1040 with serine (S).

[0032] Further, in another general aspect, the present invention provides a method for preparing 2-hydroxylated product or 4-hydroxylated product from atorvastatin including reacting at least one enzyme selected from a group consisting of wild-type CYP102A1, CYP102A1 mutants, and chimeras derived from the CYP102A1 mutants with atorvastatin,

[0033] wherein the CYP102A1 mutant has an amino acid sequence changed from that of the wild-type CYP102A1 by at least one substitution selected from a group consisting of substituting arginine (R) at the amino acid position 47 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting tyrosine (Y) at the amino acid position 51 with an amino acid selected from a group consisting of alanine, valine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting glutamic acid (E) at the amino acid position 64 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, substituting alanine (A) at the amino acid position 74 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, substituting phenylalanine (F) at the amino acid position 81 with an amino acid is selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, and tryptophan, substituting leucine (L) at the amino acid position 86 with an amino acid selected from the group consisting of alanine, valine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting phenylalanine (F) at the amino acid position 87 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, and tryptophan, substituting glutamic acid (E) at the amino acid position 143 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, substituting leucine (L) with the amino acid position 188 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, and substituting glutamic acid (E) at the amino acid position 267 with an amino acid selected from a group consisting of alanine, valine, an leucine, isoleucine, proline, methionine, phenylalanine, and tryptophan, and

[0034] the chimera derived from the CYP102A1 mutant has an amino acid sequence changed from that of the reductase domain of the CYP102A1 mutant by at least one substitution selected from a group of substituting lysine (K) at the amino acid position 474 of the of CYP102A1 mutant with threonine (T), substituting alanine (A) at the amino acid position 475 with valine (V), substituting glutamine (Q) at the amino acid position 513 with arginine (R), substituting arginine (R) at the amino acid position 526 with proline (P), substituting glutamine (Q) at the amino acid position 547 with glutamic acid (E), substituting glutamic acid (E) at the amino acid position 559 with aspartic acid (D), substituting leucine (L) at the amino acid position 590 with phenylalanine (F), substituting alanine (A) at the amino acid position 591 with serine (S), substituting aspartic acid (D) at the amino acid position 600 with glutamic acid (E), substituting valine (V) at the amino acid position 625 with leucine (L), substituting aspartic acid (D) at the amino acid position 632 with asparagine (N), substituting aspartic acid (D) at the amino acid position 638 with glutamic acid (E), substituting lysine (K) at the amino acid position 640 with alanine (A), substituting alanine (A) at the amino acid position 652 with serine (S), substituting glycine (G) at the amino acid position 661 with arginine (R), substituting threonine (T) at the amino acid position 665 with alanine (A), substituting glutamine (Q) at the amino acid position 675 with lysine (K), substituting proline (P) at the amino acid position 676 with leucine (L), substituting alanine (A) at the amino acid position 679 with glutamic acid, substituting glutamic acid (E) at the amino acid position 688 with alanine (A), substituting threonine (T) at the amino acid position 716 with alanine (A), substituting alanine (A) at the amino acid position 717 with threonine (T), substituting alanine (A) at the amino acid position 742 with glycine (G), substituting alanine (A) at the amino acid position 783 with valine (V), substituting alanine (A) at the amino acid position 796 with threonine (T), substituting lysine (K) at the amino acid position 814 with glutamic acid (E), substituting isoleucine (I) at the amino acid position 825 with methionine (M), substituting arginine (R) at the amino acid position 826 with serine (S), substituting arginine (R) at the amino acid position 837 with histidine (H), substituting glutamic acid (E) at the amino acid position 871 with asparagine (N), substituting isoleucine (I) at the amino acid position 882 with valine (V), substituting glutamic acid (E) at the amino acid position 888 with glycine (G), substituting aspartic acid (D) at the amino acid position 894 with glycine (G), substituting proline (P) at the amino acid position 895 with serine (S), substituting glycine (G) at the amino acid position 913 with serine (S), substituting glutamic acid (E) at the amino acid position 948 with lysine (K), substituting serine (S) at the amino acid position 955 with asparagine (N), substituting methionine (M) at the amino acid position 968 with valine (V), substituting glutamine (Q) at the amino acid position 971 with glutamic acid (E),) substituting methionine (M) at the amino acid position 980 with valine (V), substituting

glutamine (Q) at the amino acid position 982 with arginine (R), substituting alanine (A) at the amino acid position 1009 with aspartic acid (D), substituting aspartic acid (D) at the amino acid position 1020 with glutamic acid (E), substituting histidine (H) at the amino acid position 1022 with tyrosine (Y), substituting glutamine (Q) at the amino acid position 1023 with lysine (K) and glutamic acid (E), and substituting glycine (G) at the amino acid position 1040 with serine (S).

[0035] According to the present invention, preparation of the CYP102A1 mutants may be performed using various methods known in the art such as a deletion mutation method (Kowalski D. et al., J. Biochem., 15, 4457), a PCT method, a Kunkel method, a site-directed mutation method, a DNA shuffling, a staggered extension process (StEP), an error-prone polymerase chain reaction (PCR) method, or the like.

[0036] According to the present invention, the CYP012A1 mutant may have an amino acid sequence changed from that of the wild-type CYP102A1 by at least one substitution selected from a group consisting of substituting arginine (R) at the amino acid position 47 with leucine (L), substituting tyrosine (Y) at the amino acid position 51 with phenylalanine (F), substituting glutamic acid (E) at the amino acid position 64 with glycine (G), substituting alanine (A) at the amino acid position 74 with glycine (G), substituting phenylalanine (F) at the amino acid position 81 with isoleucine (I), substituting leucine (L) at the amino acid position 86 with isoleucine (I), substituting phenylalanine (F) at the amino acid position 87 with valine (V), substituting glutamic acid (E) at the amino acid position 143 with glycine (G), substituting leucine (L) at the amino acid position 188 with glutamine (Q), and substituting glutamic acid (E) at the amino acid position 267 with valine (V).

[0037] The most preferable CYP102A1 mutant according to the present invention may have an amino acid substitution position and substituted amino acid in the wild-type CYP102A1 selected from a group consisting of F87A, R47L/Y51E

[**0038**] A74G/F87V/188Q, R47L/L86I/L188Q, R47L/F87V/188Q,

[**0039**] R47L/F87V/L188Q/E267V, R47L/L86I/L188Q/ E267V, R47L/L86I/F87V/L188Q,

[**0040**] R47L/F87V/E143G/L188Q/E267V, R47L/E64G/F87V/E143G/L188Q/E267V,

[0041] R47L/F81I/F87V/E143G/L188Q/E267V, and

[0042] R47L/E64G/F81I/F87V/E143G/L188Q/E267V.

[0043] For example, in the CYP102A1 mutant, the amino acid substitution position and substituted amino acid in the wild-type CYP102A1 is F87A, which means that phenylalanine (F) at the amino acid position 87 in the wild-type CYP102A1 is substituted with valine (V). Hereinafter, all of the CYP102A1 mutants and the chimeras derived from the CYP102A1 mutants may also be interpreted to have the same meaning as described above.

[0044] The most preferable chimera derived from the CYP102A1 mutant according to the present invention may have an amino acid substitution position and substituted amino acid in the CYP102A1 mutant selected from a group consisting of

[0045] A475V/E559D/T665A/P676L/A679E/E688A/ A742G/K814E/R826S/R837H/E871N/I882V/E888G/ P895S/S955N/M968V/Q982R/A1009D/H1022Y/ Q1023E, [0046] A475V/E559D/T665A/A679E/E688A/A742G/ K814E/E871N/I882V/E888G/P895S/G913G/S955N/ M968V/A1009D/H1022Y/Q1023E,

[0047] K474T/A475V/A591S/D600E/V625L/D632N/ K640A/T665A/A717T/A742G/A796T/K814E/I825M/ 1882V/E888/S955N/M968V/M980V/A1009D/D1020E/ Q1023K/G1040S,

[0048] K474T/A475V/R526P/Q547E/D600E/V625 L/D638E/K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/Q971E/A1009D/ D1020E

[0049] K474T/A475V/Q513R/Q547E/D600E/V625L/ D638E/K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S 955N/M968V/A1009D/D1020E,

[0050] K474T/A475V/Q547E/D600E/V625L/D638E/ K640A/G661R/T665A/Q675K/T716A/A717T/A742G/ A783V/K814E/I825M/E871N/I882V/E888G/D894G/ E948K/S955N/M968V/A1009D/D1020E, and

[0051] K474T/A475V/Q547E/L590F/D600E/V625L/ D638E/K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/A1009D/D1020E.

[0052] Protein according to the present invention may be prepared using the methods known in the art. For example, protein may be prepared by genetic engineering techniques, peptide synthesis using solid-phase techniques (Merrifield, J. Am. Chem. Soc., 85:2149-2154 (1963)), or method of cleaving protein using peptidase.

[0053] Protein according to the present invention may be natural protein or may be prepared by a recombination of culturing cells transformed with DNA encoding CYP102A1 or mutants thereof and collecting the protein. Protein may be prepared by inserting nucleic acid molecules encoding protein according to the present invention into an expression vector, transforming the vector into a host cell, culturing the transformed host cell, and purifying protein expressed by the transformed host cell.

[0054] The vector may be, for example, plasmid, cosmid, a virus, or phage. As the host cell into which DNA in the vector is cloned or expressed, there may be a prokaryotic cell, a yeast cell, and a higher eukaryotic cell. Culture conditions such as a culture medium, a temperature, pH, and the like, may be selected by those skilled in the art without undue experiment. In general, principles, protocols, and techniques for maximizing productivity of the culture of cells may refer to Mammalian Cell Biotechnology: A Practical Approach, M. Butler, ed. (IRL Press, 1991).

[0055] The expression and cloning vector may generally include a promoter that is operationally linked to a nucleic acid sequence that encodes CYP102A1 or mutants thereof inducing the synthesis of mRNA. Various promoters that are recognized by host cells are known. A promoter suitable for a prokaryotic host cell may be a β -lactamase and lactose promoter system, alkali phosphatase, a tryptophan (trp) promoter system, and a hybrid promoter, for example, a tac promoter. In addition, the promoter used in bacterial systems may include a Shine-Dalgarno (S.D.) sequence operationally linked to DNA that encodes CYP102A1 mutants. An example of the promoter suitable for a yeast host cell may include 3-phosphooglycerate kinase or other glycosidase.

[0056] The method for preparing 2-hydroxyatorvastin or 4-hydroxylated product from atorvastatin according to the present invention may further include adding a NADPH-generating system.

[0057] The NADPH-generating system may include glucose 6-phosphate, NADP+, and yeast glucose 6-phosphate dehydrogenase, but is not limited thereto.

[0058] In the NADPH-generating system, in the case in which the wild-type CYP102A1, the CYP012A1 mutants, and the chimeras derived from the CYP102A1 mutants are reacted with atorvastatin together with each other, atorvastatin may be effectively converted into 2-hydroxylated product and 4-hydroxylated product at the same time.

[0059] In addition, the method for preparing 2-hydroxy-lated product or 4-hydroxylated product from atorvastatin according to the present invention may be performed at 0 to 40° C., and preferably, 30 to 40° C. At the time of oxidation reaction using atorvastatin as the substrate in vitro system, the catalytic activity is increased at this temperature, thereby making it possible to efficiently and selectively produce atorvastatin.

[0060] In another general aspect, the present invention provides a kit for preparing 2-hydroxylated product or 4-hydroxylated product from atorvastatin including at least one enzyme selected from a group consisting of the wild-type CYP102A1, the CYP102A1 mutants, and the chimeras derived from the CYP102A1 mutants and the NADPH-generating system,

[0061] wherein the CYP102A1 mutant includes an amino acid substitution position and substituted amino acid in the wild-type CYP102A1 selected from a group consisting of

[**0062**] F87A, R47L/Y51F, A74G/F87V/L188Q, R47L/L86I/L188Q, R47L/F87V/L188Q,

[0063] R47L/F87V/L188Q/E267V, R47L/L86I/L188Q/ E267V, R47L/L86I/F87V/L188Q,

[0064] R47L/F87V/E143G/L188Q/E267V, R47L/E64G/F87V/E143G/L188Q/E267V,

[0065] R47L/F81I/F87V/E143G/L188Q/E267V, and [0066] R47L/E64G/F81I/F87V/E143G/L188Q/E267V, and

[0067] the chimera derived from the CYP102A1 mutant includes an amino acid substitution position and substituted amino acid in the CYP102A1 mutant selected from a group consisting of

[0068] A475V/E559D/T665A/P676L/A679E/E688A/ A742G/K814E/R826S/R837H/E871N/I882V/E888G/ P895S/S955N/M968V/Q982R/A1009D/H1022Y/ Q1023E,

[0069] A475V/E559D/T665A/A679E/E688A/A742G/ K814E/E871N/I882V/E888G/P895S/G913G/S955N/ M968V/A1009D/H1022Y/Q1023E,

[0070] K474T/A475V/A591S/D600E/V625L/D632N/ K640A/T665A/A717T/A742G/A796T/K814E/I825M/ I882V/E888/S955N/M968V/M980V/A1009D/D1020E/ Q1023K/G1040S,

[0071] K474T/A475V/R526P/Q547E/D600E/V625L/ D638E/K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/Q971E/A1009D/ D1020E.

[0072] K474T/A475V/Q513R/Q547E/D600E/V625L/ D638E/K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/A1009D/D1020E, [0073] K474T/A475V/Q547E/D600V625L/D638E/ K640A/G661R/T665A/Q675K/T716A/A717T/A742G/ A783V/K814E/I825M/E871N/I882V/E888G/D894G/ E948K/S955N/M968V/A1009D/D1020E, and

[0074] K474T/A475V/Q547E/L590F/D600E/V625L/ D638E/K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/A1009D/D1020E.

[0075] The kit according to the present invention may further include a reagent required to progress the reaction.

[0076] The NADPH-generating system may include glucose 6-phosphate, NADP+, and yeast glucose 6-phosphate dehydrogenase, but is not limited thereto.

Advantageous Effects of Invention

[0077] As set forth above, the wild-type CYP102A1, the CYP102A1 mutants, and the chimeras derived from the CYP102A1 mutants according to the present invention may stably and efficiently serve as the catalyst in the reaction of converting atorvastatin into 2-hydroxylated product and 4-hydroxylated product, such that 2-hydroxylated product and 4-hydroxylated product may be environmentally-friendly and selectively prepared on a large scale.

[0078] The composition, the kit, and the method for preparing 2-hydroxylated product or 4-hydroxylated product according to the present invention includes the wild-type CYP102A1, the CYP102A1 mutants, or the chimeras derived from the CYP102A1 mutants, such that 2-hydroxylated product or 4-hydroxylated product may be economically, efficiently, and selectively prepared from atorvastatin on a large scale. Therefore, the present invention may contribute to developing novel drugs using the metabolites of atorvastatin.

BRIEF DESCRIPTION OF DRAWINGS

[0079] The above and other objects, features and advantages of the present invention will become apparent from the following description of preferred embodiments given in conjunction with the accompanying drawings, in which:

[0080] FIG. 1 shows an amino acid sequence (sequence No. 16) of a wild-type CYP102A1 according to an exemplary embodiment of the present invention;

[0081] FIG. 2 shows a nucleotide sequence (sequence No. 17) of a wild-type CYP102A1 according to another exemplary embodiment of the present invention;

[0082] FIG. 3 shows an amino acid sequence (sequence No. 18) of a wild-type CYP102A1 mutant #16 according to another exemplary embodiment of the present invention;

[0083] FIG. 4 shows a nucleotide sequence (sequence No. 19) of a wild-type CYP102A1 mutant #16 according to another exemplary embodiment of the present invention;

[0084] FIG. 5 shows an amino acid sequence (sequence No. 20) of a wild-type CYP102A1 mutant #17 according to another exemplary embodiment of the present invention;

[0085] FIG. 6 shows a nucleotide sequence (sequence No. 21 of a wild-type CYP102A1 mutant #17 according to another exemplary embodiment of the present invention;

[0086] FIG. 7 shows an amino acid sequence (sequence No. 22) of a chimera #16A1V2 derived from the wild-type CYP102A1 mutant #16 according to another exemplary embodiment of the present invention;

[0087] FIG. 8 shows a nucleotide sequence (sequence No. 23) of a chimera #16A1V2 derived from the wild-type

CYP102A1 mutant #16 according to another exemplary embodiment of the present invention;

[0088] FIG. 9 shows high-performance liquid chromatography (HPLC) chromatograms (measuring UV absorbance at 260 nm) of atorvastatin metabolites produced by human CYP3A4:

[0089] FIGS. 10A and 10B show high-performance liquid chromatography (HPLC) chromatograms (measuring UV absorbance at 260 nm) of atorvastatin metabolites produced by a CYP102A1 mutant (FIG. 10A) and a chimera (FIG. 10B) derived from a CYP102A1 mutant according to the exemplary embodiment of the present invention;

[0090] FIGS. 11A and 11B show LC-MS elution profiles of atorvastatin and metabolites thereof produced by the human CYP3A4 (FIG. 11A) and the chimera #16A1V2 derived from the CYP102A1 mutant according to the exemplary embodiment of the present invention (FIG. 11B);

[0091] FIGS. 12A to 12C show LC-MS elution profiles of atorvastatin and metabolites thereof produced by a chimera (#16A1V2) derived from the CYP102A1 mutant according to the exemplary embodiment of the present invention;

[0092] (A: 4-hydroxylated product, B: 2-hydroxylated product, C: atorvastatin)

[0093] FIG. 13 shows turnover numbers of atorvastatin oxidation using the wild-type CYP102A1, mutants and the chimera derived from the CYP102A1 mutants according to the exemplary embodiment of the present invention; and

[0094] FIG. 14 shows total turnover numbers (TTNs) of atorvastatin oxidation using chimeras derived from specific CYP102A1 mutants according to the exemplary embodiment of the present invention.

MODE FOR THE INVENTION

[0095] Hereinafter, exemplary embodiments of the present invention will be described in detail with reference to the accompanying drawings so that those skilled in the art may easily practice the present invention. However, the embodiment of the present invention has been disclosed for illustrative purposes, but the scopes of the present invention are not limited thereby.

Example 1

Construction of P450 BM3 Mutants by Site-Directed Mutagenesis

[0096] 17 site-directed mutants of CYP102A1 were prepared by the same method as a method used by Kim et al., (Drug Metab. Dispos. 35: 2166-2170, 2008b). Primers used in order to introduce BanHI/SacI restriction sites and polymerase chain reaction (PCR) primers in order to introduce mutation were shown in the following Table 1. Codons for amino acid substitution were in italics and are underlined. The PCR primers were obtained from Genotech (Daejeon, Korea). Genes encoding the CYP102A1 mutants were amplified from pCWBM3 by PCR primers designed to facilitate cloning into an expression vector pCWori (Dr. F. W. Dahlquist, University of California, Santa Barbara, Calif.) or pSE420 (Invitrogen).

[0097] Oligonucleotide assembly was performed using the 14 sets of the designed primers shown in the following Table 1. The amplified genes were cloned into the BanHI/SacI restriction sites of the PCWBM3 BanHI/SacI vector. These plasmids were transformed into *Escherichia coli* DH5αF-IQ

(Invitrogen), and this strain was also used to express the mutant CYP102A1 proteins. After mutagenesis, whether or not the desired mutations were generated was confirmed by DNA sequencing (Genotech, Daejeon, Korea).

TABLE 1

	Primers use	d to prepare mutants
Name		Sequence
BamHI forw		5' -AGC GGA TCC ATG ACA ATT AAA GAA ATG CCT C-3'
SacI rever (sequence		5' -ATC GAG CTC GTA GTT TGT AT-3'
R47L (sequence	list 3)	5' -GCG CCT GGT <u>CTG</u> GTA ACG CG-3'
Y51F (sequence	list 4)	5' -GTA ACG CGC <u>TTC</u> TTA TCA AGT-3'
E64G (sequence	list 5)	5' -GCA TGC GAT <u>GGC</u> TCA CGC TTT-3'
A74G (sequence	list 6)	5' -TA AGT CAA <u>GGC</u> CTT AAA TTT GTA CG-3'
F81I (sequence	list 7)	5' -GTA CGT GAT <u>ATT</u> GCA GGA GAC-3'
L861 (sequence	list 8)	5' -GGA GAC GGG <u>ATT</u> TTT ACA AGC T-3'
F87A (sequence	list 9)	5' -GAC GGG TTA <u>GCG</u> ACA AGC TGG-3'
F87V (sequence	list 10)	5' -GAC GGG TTA <u>GTG</u> ACA AGC TGG-3'
E143G (sequence	list 11)	5' -GAA GTA CCG <u>GGC</u> GAC ATG ACA-3'
L188Q (sequence	list 12)	5'-ATG AAC AAG <u>CAG</u> CAG CGA GCA A-3'
A264G (sequence	list 13)	5' -TTC TTA ATT <u>GGG</u> GGA CAC GTG-3'
E267V (sequence	list 14)	5' -T GCG GGA CAC <u>GTG</u> ACA ACA AGT-3'
L861/F87V (sequence	list 15)	5' -GGA GAC GGG <u>ATT</u> <u>GTG</u> ACA AGC TG-3'

Example 2

Expression and Purification of Wild-Type CYP102A1, Wild-Type CYP102A1 Mutants, and Chimeras Derived from CYP012A1 Mutant

[0098] Plasmids including genes of the Wild-type CYP102A1 (pCWBM3) and CYP102A1 mutant were transformed into *Escherichia coli* DH5αF-IQ (Kim et al., Drug Metab. Dispos. 35:2166-2170, 2008b). A culture was inoculated from a single colony into 5 ml of a Luria-Bertani medium supplemented with ampicillin (100 μg/ml) and grown at 37° C. This culture was inoculated into 250 ml of a Terrific Broth medium supplemented with ampicillin (100 μg/ml) and grown at 37° C. with shaking at 250 rpm so as to reach OD600 of about 0.8, and then gene expression was induced by the addition of isopropyl-β-D-thiogalactopyrano-

side to a final concentration of 0.5 mM. δ -Aminolevulinic acid (0.1 mM) was also added thereto. After inducing the expression, the culture was allowed to grow another 36 hours at 30° C., and then cells were harvested by centrifugation (15 min, 5000 g, 4° C.). The cell pellet was resuspended in a TES buffer solution (100 mM Tris-HCL, pH 7.6, 500 mM sucrose, 0.5 mM EDTA) and lysed by sonication (Sonicator; Misonix, Inc., Farmingdale. N.Y.). After the lysates was centrifuged at 100,000 g (90 min. 4° C.), a soluble cytosolic fraction was collected and used for the activity assay. The soluble cytosolic fraction was dialyzed from a 50 mM potassium phosphate buffer (pH 7.4) and stored at -80° C. The cytosolic fraction was used within 1 month of manufacture.

[0099] The CYP102A1 concentrations were determined from CO-difference spectra using ϵ =91 mM/cm (Omura and

Sato. J. Biol. Chem. 239:2370-2378, 1964). For all of the wild-types and mutants, a typical culture yielded 300 to 700 nM P450. The expression level of wild-type CYP102A1 and the mutants thereof were in the range of 1.0 to 2.0 nmol P450/mg cytosolic protein.

[0100] Several mutants with high catalytic activity for some substrates in human were selected among the prepared mutants, and the amino acid substitution sites in the mutants were shown in Tables 2 and 3.

REFERENCES

[0101] Carmichael and Wong. Eur. J. Biochem. 268:3117-3125, 2001; Li et al., Appl. Environ. Microbiol. 67:5735-5739, 2001; van Vugt-Lussenburg et al., J. Med. Chem. 50:455-461, 2007

TABLE 2

	CYP102A1 mutants used in the preso	ent invention
Abbreviations	BM3 wild type and mutants	Ref
WT	BM3 wild type	Carmichael and Wong, 2001
Mutant #1	F87A	Carmichael and Wong, 2001
Mutant #2	A264G	Carmichael and Wong, 2001
Mutant #3	F87A/A264G	Carmichael and Wong, 2001
Mutant #4	R47L/Y51F	Carmichael and Wong, 2001
Mutant #5	R47L/Y51F/A264G	Carmichael and Wong, 2001
Mutant #6	R47L/Y51F/F87A	Carmichael and Wong, 2001
Mutant #7	R47L/Y51F/F87A/A264G	Carmichael and Wong, 2001
Mutant #8	A74G/F87V/L188Q	Li et al., 2001
Mutant #9	R47L/L86I/L188Q	Kim et al., 2008b
Mutant #10	R47L/F87V/L188Q	van Vugt-Lussenburg et al., 2007
Mutant #11	R47L/F87V/L188Q/E267V	van Vugt-Lussenburg et al., 2007
Mutant #12	R47L/L86I/L188Q/E267V	Kim et al., 2008b
Mutant #13	R47L/L86I/F87V/L188Q	van Vugt-Lussenburg et al., 2007
Mutant #14	R47L/F87V/E143G/L188Q/E267V	Kim et al., 2008b
Mutant #15	R47L/E64G/F87V/E143G/L188Q/E267V	Kim et al., 2008b
Mutant #16	R47L/F81I/F87V/E143G/L188Q/E267V	Kim et al., 2008b
Mutant #17	R47L/E64G/F81I/F87V/E143G/L188Q/E26	7V van Vugt-Lussenburg et al., 2007

TABLE 3

	CYP102A1 natural variants used in the present invention CYP102A1 Variants											
	Mutated Amino acid	Change of Nucleotide	*2	*3	*4	*5	*6	*7	*8	*9	QMB1551	
	T2P	4A > C									+	
Heme	V27I	79G > A	+		+		+	+	+	+	+	
domain	A29T	85G > A	+		+		+	+	+	+	+	
	V128I	382G > A	+		+	+	+	+	+	+	+	
	A136T	406G > A	+		+		+	+	+	+	+	
	E208D	624A > C				+						
	A222T	664G > A									+	
	A296T	886G > A	+		+							
	D370E	1110C > A	+		+							
	K453Q	1357A > C				+	+	+	+	+	+	
	T464R	1392T > A				+	+	+	+	+	+	
	V471E	1413A > G				+	+	+	+	+	+	
Reductase	K474T	1422G > C				+	+	+	+	+	+	
domain	A475V	1424C > T	+	+	+	+	+	+	+	+	+	
	Q513R	1539G > A						+				
	R526P	1578C > T					+					
	Q547E	1639C > G					+	+	+	+	+	
	E559D	1677A > C	+	+	+							
	L590F	1794C > A								+		
	A591S	1771G > T				+						
	D600E	1800C > A				+	+	+	+	+	+	
	V625L	1873G > T				+	+	+	+	+	+	
	D632N	1894G > A				+						
	D638E	1914T > A					+	+	+	+	+	

TABLE 3-continued

CYP10	02A1 natural va CY.	riants P102 <i>A</i>				sent i	nven	tion		
Mutated Amino acid	Change of Nucleotide	*2	*3	*4	*5	*6	*7	*8	*9	QMB1551
K640A	1920A > T				+	+	+	+	+	+
A652S	1954G > T									+
G661R	1981G > C					+	+	+	+	+
T665A	1993A > G	+	+	+	+	+	+	+	+	+
Q675K	2023C > A					+	+	+	+	+
P676L	2027C > T	+	+							
A679E	2036C > A	+	+	+						
E688A	2063A > C	+	+	+						
T716A	2146A > G					+	+	+	+	+
A717T	2149G > A				+	+	+	+	+	+
A742G	2225C > G	+	+	+	+	+	+	+	+	+
A783V	2348C > T					+	+	+	+	+
A796T	2386G > A				+					
K814E	2440A > G	+	+	+	+	+	+	+	+	+
I825M	2474A > G				+	+	+	+	+	+
R826S	2476C > A	+	+							
R837H	2510G > A	+	+							
E871N	2613G > T	+	+	+		+	+	+	+	+
I882V	2644A > G	+	+	+	+	+	+	+	+	+
E888G	2663A > G	+	+	+	+	+	+	+	+	+
D894G	2681A > G					+	+	+	+	+
P895S	2683C > T	+	+	+						
G913S	2739C > T			+						
E948K	2842G > A					+	+	+	+	+
S955N	2864G > A	+	+	+	+	+	+	+	+	+
M968V	2904G > A	+	+	+	+	+	+	+	+	+
Q971E	2911C > G					+				
M980V	2938A > G				+					
Q982R	2945A > G	+	+							
À1009D	3026C > A	+	+	+	+	+	+	+	+	+
D1020E	3060C > A				+	+	+	+	+	+
H1022Y	3066C > T	+	+	+		•				•
Q1023K	3067C > G	•	•	•	+					
Q1023E	3067C > A	+	+	+	-					
G1040S	3118G > A			•	+					

[0102] In addition, a chimeric protein of selective CYP102A1 mutants was constructed by fusing heme domains of the prepared CYP102A1 mutants of Tables 2 and 3 to reductase domains of the natural variants of the wild-type CYP102A1

[0103] In order to clone the chimeric protein of the selective CYP102A1 mutant prepared by fusing the heme domain and the reductase domain to each other, the chimeric protein was cloned into the expression vector pCW vector prepared using BanHI/SacI and SacI/XhoI.

[0104] Plasmids including genes of the chimeric protein of the CYP102A1 mutant were transformed into *Escherichia coli* DH5 α F-IQ (Kim et al. Protein Expr. Purif. 57:188-200, 2008). A culture was inoculated from a single colony into 5 ml of a Luria-Bertani medium supplemented with ampicillin (100 μ g/ml) and grown at 37° C. This culture was inoculated into 250 ml of a Terrific Broth medium supplemented with ampicillin (100 μ g/ml) and grown at 37° C. with shaking at 250 rpm so as to reach OD600 of about 0.8, and then gene expression was induced by the addition of isopropyl- β -D-thiogalactopyranoside to a final concentration of 0.5 mM.

[0105] δ -Aminolevulinic acid (0.1 mM) was also added thereto. After inducing of the expression, the culture was allowed to grow another 36 hours at 30° C., and then cells were harvested by centrifugation (15 min, 5000 g, 4° C.). The cell pellet was resuspended in a TES buffer solution (100 mM Tris-HCL, pH 7.6, 500 mM sucrose, 0.5 mM EDTA) and

lysed by sonication (Sonicator. Misonix. Inc., Farmingdale. N.Y.). After the lysates was centrifuged at $100,000\,\mathrm{g}$ ($90\,\mathrm{min}$, $4^\circ\,\mathrm{C.}$), a soluble cytosolic fraction was collected and used for the activity assay. The soluble cytosolic fraction was dialyzed from a $50\,\mathrm{mM}$ potassium phosphate buffer (pH 7.4) and stored at $-80^\circ\,\mathrm{C.}$ The cytosolic fraction was used within 1 month of manufacture.

[0106] The CYP102A1 concentrations were determined from CO-difference spectra using ϵ =91 mM/cm (Omura and Sato, J. Biol. Chem. 239:2379-2385 1964). For the chimeras derived from CYP102A1, a typical culture yielded 300 to 700 nM P450. The expression levels of the chimeras derived from the CYP102A1 mutant were in the range of 1.0 to 2.0 nmol P450/mg cytosolic protein.

[0107] Several chimeras with high catalytic activity for some substrates in a human were selected among the chimeras prepared from the CYP102A1 mutants, and the amino acid substitution sites in each chimera were shown in Table 4 (Kang et al., AMB Express, 1:1, 2011).

[0108] Hereinafter, the chimeras derived from the CYP102A1 mutants used in this experiment were called as follows.

[0109] In the present invention, the terms chimera #16A1V2 of the mutants means a chimera derived from a CYP102A1 mutant #16 prepared by fusing the heme domains of the mutant #16 in Table 2 to V2 reductase domain of the following Table 4.

TABLE 4

	CYP102A1 natural variants used in the present invention	
Abbreviations	Natural variants	Ref
variant2(V2)	A475V/E559D/T665A/P676L/A679E/E688A/A742G/K814E/ R826S/R837H/E871N/I882V/E888G/P895S/S955N/M968V/ O982R/A1009D/H1022Y/O1023E	Kang et al. 2011
variant3(V3)	Q922NA1009D/H1022 I/Q1023E A475V/E559D/T665A/P676L/A679E/E688A/A742G/K814E/ R826S/R837H/E871N/I882V/E888G/P895S/S955N/M968V/ Q982R/A1009D/H1022Y/Q1023E	Kang et al. 2011
variant4(V4)	A475V/E559D/T665A/A679E/E688A/A742G/K814E/E871N/ 1882V/E888G/P895S/G913G/S955N/M968V/A1009D/H1022Y Q1023E	Kang et al. 2011
variant5(V5)	K474T/A475V/A5918/D600E/V625L/D632N/K640A/T665A/ A71.7T/A742G/A796T/K814E/I825M/I882V/E888/S955N/ M968V/M980V/A1009D/D1020E/Q1023E/G1040S	Kang et al. 2011
variant6(V6)	K474T/A475V/R526P/Q547E/D600E/V625L/D638E/K640A/ G661R/T665A/Q675K/T71GA/A717T/A742G/A783V/K814E/ I825M/E871N/I882V/E888G/D894G/E948K/S955N/M968V/	Kang et al. 2011
variant7(V7)	Q971E/A1009D/D1020E K474T/A475V/Q513R/Q547E/D600E/V625L/D638E/K640A/ G661R/T665A/Q675K/T716A/A717T/A742G/A783V/K814E/ I825M/E871N/I882V/E888G/D894G/E948K/S955N/M968V/ A1009D/D1020E	Kang et al. 2011
variant8(V8)	K474T/A475V/Q547E/D600E/V625L/D638E/K640A/G661R/ T665A/Q675K/T716A/A717T/A742G/A783V/K814E/I825M/ E87IN/I882V/E888G/D894G/E948K/S955N/M968V/A1009D/ D1020E	Kang et al. 2011
variant9(V9)	K474T/A475V/Q547E/L590F/D600E/V625L/D638E/K640A/ G661R/T665A/Q675K/T716A/A717T/A742G/A783V/K814E/ I825M/E871N/I882V/R888G/D894G/E948K/S955N/M968V/ A1009D/D1U20E	Kang et al. 2011

Example 3

Oxidation of Atorvastatin by Wild-Type CYP102A1, Wild-Type CYP102A1 Mutants, and Chimeras Derived from CYP102A1 Mutant

[0110] was examined whether the wild-type CYP102A1, the CYP102A1 mutants, and the chimeras derived from the CYP1.02A1 mutants may oxidize atorvastatin. Typical steady-state reactions was performed by adding 50 pmol CYP102A1 and 80 μ M substrate to 0.25 ml of 100 mM potassium phosphate buffer solution (pH 7.4). In order to initiate reactions, the NADPH-generating system was added thereto (final concentrations: 10 mM glucose 6-phosphate, 0.5 mM NADP+, and 1 IU yeast glucose 6-phosphate per ml). A stock solution of atorvastatin (20 mM) was prepared in DMSO and diluted into the enzyme reaction solution to have a final organic solvent concentration of <1% (v/v).

[0111] In order to measure human CYP3A4 activity, 50 pmol P450, 100 pmol NADPH-P450 reductase (CPR), 100 pmol cytochrome b5, and 45 μ M L- α -dilauroyl-sn-glycero-3-phosphocholine (DLPC) were used instead of 50 pmol CYP102A1. After the reaction solution was reacted for 30 minutes at 37° C., the reaction was terminated with 2-fold of ice-cold dichloromethane.

[0112] (1) HPLC Analysis

[0113] After centrifugation of the reaction mixture, a supernatant was removed and a solvent was evaporated under nitrogen gas and analyzed using HPLC. A sample (30 ul) was injected into Gemini C18 column (4.6 mm×150 mm, 5 um. Phenomenex. Torrance, Calif.). As a mobile phase A, water containing 0.1% formic acid/acetonitrile (80/20, v/v) was used, and as a mobile phase B, acetonitrile/0.1% formic acid (90/10, v/v) was used. The mobile phase A/B (70/30, v/v) was

flowed at a rate of 1 ml·min⁻¹ using a gradient pump (LC-20AD, Shimadzu, Kyoto, Japan). Elution solutions were detected by UV at 260 nm.

[0114] In order to examine whether or not CYP102A1 (P450 BM3) may oxidize atorvastatin, the abilities of the wild-type CYP102A1 (P450 BM3), the mutants thereof, and the chimeras derived from the CYP102A1 mutants to oxidize atorvastatin were measured at a fixed substrate concentration $(80 \, \mu M)$.

[0115] The metabolites of atorvastatin prepared by the human CYP3A4, the bacterial CYP102A1 mutant (#16 in Table 2), and the chimera (#16A1V3) derived from the CYP102A1 were examined using HPLC chromatograms (measuring UV absorbance at 260 nm).

[0116] Peaks were confirmed by comparing with retention times of peaks of the metabolites prepared by human CYP3A4 and CYP2C9. The substrate and two main metabolites, that is, 2-hydroxylated product and 4-hydroxylated product were shown.

[0117] As a result, it might be appreciated that retention times of the peaks of the metabolites exactly coincide with those of the standard 4-OH atorvastatin and 2-OH atorvastatin as shown in FIGS. 9 to 10B.

[0118] (2) LC-MS Analysis

[0119] In order to identify atorvastatin metabolites produced the wild-type CYP102A1 mutants and the chimeras derived from by CYP102A1 mutants, LC-MS analysis was conducted by comparing LC profiles and fragmentation patterns of atorvastatin and metabolites thereof.

[0120] The wild-type CYP102A1 mutants and human CYP3A4 were incubated with $80\,\mu\text{M}$ of atorvastatin at 37° C. for 30 minutes in the presence of an NADPH-generating system. Reactions were terminated by the addition of 2-fold ice-cold CH₂Cl₂. After centrifugation of the reaction mixture,

a supernatant was removed and an organic solvent layer was evaporated under nitrogen. The reactant was reconstituted into 100 μ l of a mobile phase by vortex mixing and sonication for 20 sec. An aliquot (10 μ l) of the prepared solution was injected into the LC column.

[0121] LC-MS analysis was carried out on Shimadzu LCMS-2010 EV system (Shimadzu Corporation, Japan) having LCMS solution software by electro spray ionization in a positive mode. In a Shim-pack VP-ODS column (250 mm×2.0 mm i.d., Shimadzu Corporation, Japan) water containing 0.1% formic acid/acetonitrile (80/20, v/v) was used as a mobile phase A, and acetonitrile/0.1% formic acid (90/10, v/v) was used as a mobile phase B. The mobile phase A/B (70/30, v/v) was separated using a gradient pump (LC-20AD, Shimadzu. Kyoto, Japan) at a flow rate of 0.16 ml/min. In order to identify the metabolites, mass spectra were recorded by electro spray ionization in a negative mode. Interface and detector voltages are 4.4 kV and 1.5 kV, respectively. Nebulization gas flow was set at 1.5 ml/min. and interface, curve desolvation line (CDL), and heat block temperatures were 250, 230, and 200° C., respectively.

[0122] As a result, it might be appreciated that in mass spectra of the reaction samples, peaks were observed at 7.183 min (4-OH atorvastatin), 19.583 min (2-OH atorvastatin), and 21.450 min (atorvastatin) as shown in total ion current (TIC) profiles of the metabolites prepared by the human CYP3A4 (A) and the chimera #16A1V2 (B) derived from the CYP102A1 mutant of FIG. 11.

[0123] Further, as shown in FIGS. 12A to 12C, the peaks in mass spectra of 4-hydroxylated products (A), 2-hydroxylated products (B), and atorvastatin products (C) by the chimera #16A1V2 derived from the CYP102A1 mutant were observed at 573, 573, and 557, respectively, when calculated as [M-H]⁻.

[0124] Based on the results of LC-MS analysis of the reactants, it might be appreciated that the CYP102A1 mutants and the chimeras derived from the CYP102A1 mutants produce 4-hydroxylated or 2-hydroxylated product from atorvastatin. The retention time and fragmentation pattern of the metabolites produced by the CYP102A1 mutants and the chimeras derived from the CYP102A1 mutants were exactly matched to those of authentic metabolites produced by human CYP3A4.

[0125] (3) Determination of Turnover Number

[0126] In order to recognize production rate of atorvastatin oxides by wild-type CYP102A1, CYP102A1 mutants, and chimeras derived from the CYP102A1 mutants, the turnover number was determined in the reaction using $80~\mu M$ statin.

[0127] The term "turnover number" means the number of substrate molecules that a molecule of an enzyme may convert into products per minute and indicates conversion frequency.

[0128] The production rate of 4-hydroxylated metabolite was determined by HPLC as described above.

[0129] As shown in FIG. 13, it might be appreciated that three kinds of mutants (#15, #16, and #17 in Table 2) and five kinds of chimeras (#16A1V2, #16A1V3, #17A1V2, #17A1V3, and #17A1V8) derived from the mutants have high turnover number as the results of measuring the turnover numbers of 17 kinds of mutants and 7 kinds of chimeras derived from the mutants in oxidation of atorvastatin (producing the metabolites of atorvastatin).

[0130] Particularly, it might be appreciated that the chimeras #16A1V2 and #17A1V2 derived from the mutants have the same activity as that of the human CYP3A4.

[0131] In order to recognize production rate of atorvastatin metabolites by the CYP102A1 mutant (#16 in Table 2) and the chimeras (#16A1V2 and #17A1V2) derived from the CYP102A1 mutants, total turnover numbers (TTNs; mol product/mol catalyst) were determined in reactions using total 240 μ M atorvastatin.

[0132] The term "total turnover number (TTN)" means the number of substrate molecules converted into metabolites by enzymes for the total reaction time.

[0133] The total turnover numbers (TTNs) were determined by comparing the results under three conditions. First, the reaction was performed by adding a NADPH-generating system at 37° C. for 1 hour in the presence of 80 μM substrate. In addition, second, after reaction was performed for 1 hour in the presence of 80 μM substrate, 80 μM substrate was additionally added to the reaction mixture, and the reaction was performed for 1 hour in the presence of 80 μM substrate, 80 μM substrate was additionally added to the reaction mixture, and the reaction was further performed for 1 hour. Then, 80 μM substrate was additionally added to the reaction mixture, and the reaction was further performed for 1 hour. Then, 80 μM substrate was additionally added to the reaction mixture, and the reaction was further performed for 2 hours.

[0134] The production rate of the atorvastatin metabolites was determined using HPLC. The enzyme capable of most efficiently producing a large amount of metabolites in vitro may be selected by comparing the results according to concentration of the substrate and reaction time using mutants or chimeras derived from the mutants having higher activity based on experimental results of the turnover number.

[0135] As a result, the total turnover numbers (TTNs; mol product/mol catalyst) were in a range of 31 to 83 as shown in FIG. 14.

[0136] Particularly, when the chimeras #16A1V2 and #17A1V2 derived from CYP102A1 mutants having high activity were reacted for 4 hours, it might be appreciated that #16A1V2 has activity higher than that of the human CYP3A4.

[0137] The production of metabolites of atorvastatin by chemical synthesis has never been reported up to now. Therefore, it may be an alternative to chemical synthesis of the target metabolites in the Examples of the present invention to use CYP102A1 enzymes, that is, CYP102A1 mutants and the chimeras derived from the CYP102A1 mutants to generate the metabolites of atorvastatin according to the present invention

[0138] According to the present invention, it might be appreciated that bacterial CYP102A1 enzymes of the Examples catalyze the same reaction as that of the human CYP3A4 to produce 4-OH product and 2-OH product, which are the human metabolites.

[0139] In addition, it might be appreciated that the wild-type CYP102A1 mutants and the chimeras derived from the CYP102A1 mutants catalyze oxidation of atorvastatin, which is the human P450 substrate, and produces 4-hydroxylated product and 2-hydroxylated product, which are the main metabolites produced by the human CYP3A4, from atorvastatin.

[0140] Further, it may be appreciated that the wild-type CYP102A1 mutants and the chimeras derived from the CYP102A1 mutants according to the present invention may efficiently produce the human metabolites from atorvastatin,

these metabolites may be used to estimate effect, toxicity, and pharmacokinetics of drugs, or the like in a process of developing the drugs, and used to prepare human metabolite derivatives capable of serving as a lead compound of developing the drug.

SEQUENCE LISTING FREE TEXT

[0141] SEQ. ID. NO: 1 to 15 are primer sequence

[0142] SEQ. ID. NO: 16 is an amino acid sequence of a wild-type CYP102A1

[0143] SEQ. ID. NO: 17 is a nucleotide sequence of a wild-type CYP102A1

[0144] SEQ. ID. NO: 18 is an amino acid sequence of a wild-type CYP102A1 mutant #16

[0145] SEQ. ID. NO: 19 is a nucleotide sequence of a wild-type CYP102A1 mutant #16

[0146] SEQ. ID. NO: 20 is an amino acid sequence of a wild-type CYP102A1 mutant #17

[0147] SEQ. ID. NO: 21 is a nucleotide sequence of a wild-type CYP102A1 mutant #17

[0148] SEQ. ID. NO: 22 is an amino acid sequence of a chimera #16A1V2 derived from the wild-type CYP102A1 mutant #16

[0149] SEQ. ID. NO: 23 is a nucleotide sequence of a chimera #16A1V2 derived from the wild-type CYP102A1

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Leu 785	Glu	Lys	Gln	Ala	Tyr 790	Lys	Glu	Gln	Val	Leu 795	Ala	Lys	Arg	Leu	Thr 800
Met	Leu	Glu	Leu	Leu 805	Glu	Lys	Tyr	Pro	Ala 810	Cys	Glu	Met	ГÀа	Phe 815	Ser
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Phe	Ile	Ser	Thr	Pro 885	Gln	Ser	Glu	Phe	Thr 890	Leu	Pro	Lys	Asp	Pro 895	Glu
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Leu His Thr Ala Phe Ser Arg Met Pro Asn Gln Pro Lys Thr Tyr Val 965 970 975
Gln His Val Met Glu Gln Asp Gly Lys Leu Ile Glu Leu Leu Asp 980 985 990
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Ala Val Glu Ala Thr Leu Met Lys Ser Tyr Ala Asp Val His Gln 1010 1015 1020
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### 1855	Val		Ser	Gly	Glu	Ala		Ser	Gly	Tyr	Gly		Tyr	Lys	Gly	Ile	
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Gly Glu Ala His Leu Tyr Phe Gly Cys Arg Ser Pro His Glu Asp Tyr 930 955 Leu Tyr Gln Glu Glu Leu Glu Asn Ala Gln Ser Glu Gly Ile Ile Thr 945 950 960 Leu His Thr Ala Phe Ser Arg Met Pro Asn Gln Pro Lys Thr Tyr Val 965 970 975 Gln His Val Met Glu Gln Asp Gly Lys Lys Leu Ile Glu Leu Leu Asp 980 985 990 Gln Gly Ala His Phe Tyr Ile Cys Gly Asp Gly Ser Gln Met Ala Pro 1005 Ala Val Glu Ala Thr Leu Met Lys Ser Tyr Ala Asp Val His Gln 1010 1015 Ala Val Glu Ala Asp Ala Arg Leu Trp Leu Gln Gln Leu Glu Glu 1025 1030 1035 Lys Gly Arg Tyr Ala Lys Asp Val Trp Ala Gly 1045	Thi	Pro	Leu			Val	Gly	Pro		Thr	Gly	Val	Ala		Phe	Arg	
Leu Tyr Gln Glu Glu Leu Glu Asn Ala Gln Ser Glu Gly Ile Ile Thr 945 950 955 960 Leu His Thr Ala Phe Ser Arg Met Pro Asn Gln Pro Lys Thr Tyr Val 965 970 970 975 Gln His Val Met Glu Gln Asp Gly Lys Leu Ile Glu Leu Leu Asp 980 985 990 Gln Gly Ala His Phe Tyr Ile Cys Gly Asp Gly Ser Gln Met Ala Pro 995 1000 1005 Ala Val Glu Ala Thr Leu Met Lys Ser Tyr Ala Asp Val His Gln 1010 1015 1030 1035 Lys Gly Arg Tyr Ala Lys Asp Val Trp Ala Gly 1040 1045 <pre> </pre> <a <="" href="https://doi.org/10.1006/j.com/district/" td=""><td>Gly</td><td>7 Phe</td><td></td><td>Gln</td><td>Ala</td><td>Arg</td><td>-</td><td></td><td>Leu</td><td>Lys</td><td>Glu</td><td>Gln</td><td>_</td><td>Gln</td><td>Ser</td><td>Leu</td><td></td>	Gly	7 Phe		Gln	Ala	Arg	-		Leu	Lys	Glu	Gln	_	Gln	Ser	Leu	
945 950 955 960 Leu His Thr Ala Phe Ser Arg Met Pro Asn Gln Pro Lys Thr Tyr Val 965 970 975 Gln His Val Met Glu Gln Asp Gly Lys Lys Leu Ile Glu Leu Leu Asp 980 985 980 985 Gln Gly Ala His Phe Tyr Ile Cys Gly Asp Gly Ser Gln Met Ala Pro 995 1000 1005 Ala Val Glu Ala Thr Leu Met Lys Ser Tyr Ala Asp Val His Gln 1010 1015 1020 Val Ser Glu Ala Asp Ala Arg Leu Trp Leu Gln Gln Leu Glu Glu 1025 1030 1035 Lys Gly Arg Tyr Ala Lys Asp Val Trp Ala Gly 1045 <pre> </pre> <pre> </pre> <pre> <pre> </pre> <pre> </pre> <pre> <pre> <pre> </pre> <pre> <pre> <pre> <pre> </pre> <pre> <pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre>	Gly		Ala	His	Leu	Tyr		Gly	Cys	Arg	Ser		His	Glu	Asp	Tyr	
Gln His Val Met Glu Gln Asp Gly Lys Lys Leu Ile Glu Leu Leu Asp 980 985 990 Gln Gly Ala His Phe Tyr Ile Cys Gly Asp Gly Ser Gln Met Ala Pro 1000 1005 Ala Val Glu Ala Thr Leu Met Lys Ser Tyr Ala Asp Val His Gln 1010 1015 1020 Val Ser Glu Ala Asp Ala Arg Leu Trp Leu Gln Gln Leu Glu Glu 1025 1030 1035 Lys Gly Arg Tyr Ala Lys Asp Val Trp Ala Gly 1040 1045 <pre> </pre> <pre> </pre> <pre> </pre> <pre> </pre> <pre> </pre> <pre> </pre> <pre> <pre> <pre> </pre> <pre> <pre> </pre> <pre> <pre> <pre> <pre> </pre> <pre> <pre> <pre> <pre> <pre> <pre> <pre> </pre> <pre> <pr< td=""><td></td><td>_</td><td>Gln</td><td>Glu</td><td>Glu</td><td></td><td>Glu</td><td>Asn</td><td>Ala</td><td>Gln</td><td></td><td>Glu</td><td>Gly</td><td>Ile</td><td>Ile</td><td></td><td></td></pr<></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre></pre>		_	Gln	Glu	Glu		Glu	Asn	Ala	Gln		Glu	Gly	Ile	Ile		
Gln Gly Ala His Phe Tyr Ile Cys Gly Asp Gly Ser Gln Met Ala Pro 995 Ala Val Glu Ala Thr Leu Met Lys Ser Tyr Ala Asp Val His Gln 1010 Val Ser Glu Ala Asp Ala Arg Leu Trp Leu Gln Gln Leu Glu Glu 1025 Lys Gly Arg Tyr Ala Lys Asp Val Trp Ala Gly 1040 C210 > SEQ ID NO 19 C211 > LENGTH: 3150 C212 > TYPE: DNA C213 > ORGANISM: Artificial sequence C220 > FEATURE: C223 > OTHER INFORMATION: CYP102Al mutant #16 C400 > SEQUENCE: 19 atgacaatta aagaaatgcc tcagccaaaa acgtttggag agcttaaaaa tttaccgtta 60 ttaaacacag ataaaccggt tcaagctttg atgaaaattg cggatgaatt aggagaaatc 120 tttaaattcg aggcgcctgg tcttgtaacg cgctacttat caagtcagcg tctaattaaa 180 gaagcatgcg atgaatcacg ctttgataaa aacttaagtc aagcgttaa atttgtacgt 240 gatattgcag gagacggtt agttacaagc tggacgatg aaaaaaattg gaaaaaagcg 300 cataatatct tacttccaag cttcagtcag caggcaatga aaggctatca tgcgatgatt 420 gatattgcag gagacggtt tgttcaaaag tggagcgtc taaatgcag tgagcatatt 420 gacgataccg gagacatgac tgttcaaaag tggagacgtc taaatgcag tgagcatatt 420 gaagtaccgg gagacatgac acgtttaacag cttgatacaa ttggtctttg cggctttaac 480	Leu	ı His	Thr	Ala		Ser	Arg	Met	Pro		Gln	Pro	ГÀв	Thr		Val	
Ala Val Glu Ala Thr Leu Met Lys Ser Tyr Ala Asp Val His Gln 1010 Val Ser Glu Ala Asp Ala Arg Leu Trp Leu Gln Gln Leu Glu Glu 1025 Lys Gly Arg Tyr Ala Lys Asp Val Trp Ala Gly 1035 C210> SEQ ID NO 19 C211> LENGTH: 3150 C212> TYPE: DNA C213> ORGANISM: Artificial sequence C220> FEATURE: C223> OTHER INFORMATION: CYP102Al mutant #16 C400> SEQUENCE: 19 atgacaatta aagaaatgc tcagccaaaa acgtttggag agcttaaaaa tttaccgtta 60 ttaaaccacag ataaaccggt tcaagctttg atgaaaattg cggatgaatt aggagaaatc 120 tttaaattcg aggcgcttgg tcttgtaacag cgctacttat caagtcagcg tctaattaaa 180 gaagcatgcg atgaatcacg ctttgataaa aacttaagtc aagcgcttaa atttgtacgt 240 gatattgcag gagacgggtt agttacaagc tggacgcatg aaaaaaaattg gaaaaaagcg 300 cataatatct tacttccaag cttcagtcag caggcaatga aaggctatca tgcgatgatg 360 gtcgatatcg ccgtgcagct tgttcaaaag ttggagcgtc taaatgcaga tgagcatatt 420 gaagtaccgg gagacatgac acgtttaacg cttgatacaa ttggtctttg cggctttaac 480	Glr	n His	Val			Gln	Asp	Gly		Lys	Leu	Ile	Glu		Leu	Asp	
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Ala	Lys	Met	His 660	Gly	Ala	Phe	Ser	Ala 665	Asn	Val	Val	Ala	Ser 670	Lys	Glu
Leu	Gln	Gln 675	Leu	Gly	Ser	Glu	Arg 680	Ser	Thr	Arg	His	Leu 685	Glu	Ile	Ala
Leu	Pro 690	Lys	Glu	Ala	Ser	Tyr 695	Gln	Glu	Gly	Asp	His 700	Leu	Gly	Val	Ile
Pro 705	Arg	Asn	Tyr	Glu	Gly 710	Ile	Val	Asn	Arg	Val 715	Thr	Ala	Arg	Phe	Gly 720
Leu	Asp	Ala	Ser	Gln 725	Gln	Ile	Arg	Leu	Glu 730	Ala	Glu	Glu	Glu	Lys 735	Leu
Ala	His	Leu	Pro 740	Leu	Gly	Lys	Thr	Val 745	Ser	Val	Glu	Glu	Leu 750	Leu	Gln
Tyr	Val	Glu 755	Leu	Gln	Asp	Pro	Val 760	Thr	Arg	Thr	Gln	Leu 765	Arg	Ala	Met
Ala	Ala 770	Lys	Thr	Val	CAa	Pro 775	Pro	His	Lys	Val	Glu 780	Leu	Glu	Ala	Leu
Leu 785	Glu	Lys	Gln	Ala	Tyr 790	Lys	Glu	Gln	Val	Leu 795	Ala	Lys	Arg	Leu	Thr 800
Met	Leu	Glu	Leu	Leu 805	Glu	Lys	Tyr	Pro	Ala 810	Сув	Glu	Met	Glu	Phe 815	Ser
Glu	Phe	Ile	Ala 820	Leu	Leu	Pro	Ser	Ile 825	Ser	Pro	Arg	Tyr	Tyr 830	Ser	Ile
Ser	Ser	Ser 835	Pro	His	Val	Asp	Glu 840	Lys	Gln	Ala	Ser	Ile 845	Thr	Val	Ser
Val	Val 850	Ser	Gly	Glu	Ala	Trp 855	Ser	Gly	Tyr	Gly	Glu 860	Tyr	Lys	Gly	Ile
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Phe	Val	Ser	Thr	Pro 885	Gln	Ser	Gly	Phe	Thr 890	Leu	Pro	Lys	Asp	Ser 895	Glu
Thr	Pro	Leu	Ile 900	Met	Val	Gly	Pro	Gly 905	Thr	Gly	Val	Ala	Pro 910	Phe	Arg
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Leu 945	Tyr	Gln	Glu	Glu	Leu 950	Glu	Asn	Ala	Gln	Asn 955	Glu	Gly	Ile	Ile	Thr 960
Leu	His	Thr	Ala	Phe 965	Ser	Arg	Val	Pro	Asn 970	Gln	Pro	Lys	Thr	Tyr 975	Val
Gln	His	Val	Met 980	Glu	Arg	Asp	Gly	Lys 985	Lys	Leu	Ile	Glu	Leu 990	Leu	Asp

Gln Gly Ala His Phe Tyr Ile Cys Gly Asp Gly Ser Gln Met Ala Pro Asp Val Glu Ala Thr Leu Met Lys Ser Tyr Ala Asp Val Tyr Glu 1010 Val Ser Glu Ala Asp Ala Arg Leu Trp Leu Gln Gln Leu Glu Glu 1025 1030 Lys Gly Arg Tyr Ala Lys Asp Val Trp Ala Gly <210> SEQ ID NO 23 <211> LENGTH: 3150 <212> TYPE: DNA <213> ORGANISM: Artificial sequence <220> FEATURE: <223 > OTHER INFORMATION: chimera #16A1V2 <400> SEQUENCE: 23 atgacaatta aagaaatgcc tcagccaaaa acgtttggag agcttaaaaa tttaccgtta 60 120 ttaaacacag ataaaccggt tcaagctttg atgaaaattg cggatgaatt aggagaaatc tttaaattcg aggcgcctgg tcttgtaacg cgctacttat caagtcagcg tctaattaaa 180 gaagcatgcg atgaatcacg ctttgataaa aacttaagtc aagcgcttaa atttgtacgt 240 gatattgcag gagacgggtt agttacaagc tggacgcatg aaaaaaattg gaaaaaagcg 300 cataatatct tacttccaag cttcagtcag caggcaatga aaggctatca tgcgatgatg 360 gtcgatatcg ccgtgcagct tgttcaaaag tgggagcgtc taaatgcaga tgagcatatt 420 gaagtaccgg gagacatgac acgtttaacg cttgatacaa ttggtctttg cggctttaac 480 tategettta acagetttta eegagateag eeteateeat ttattacaag tatggteegt 540 gcactggatg aagcaatgaa caagcagcag cgagcaaatc cagacgaccc agcttatgat 600 gaaaacaagc gccagtttca agaagatatc aaggtgatga acgacctagt agataaaatt 660 attgcagatc gcaaagcaag cggtgaacaa agcgatgatt tattaacgca tatgctaaac 720 ggaaaagatc cagaaacggg tgagccgctt gatgacgaga acattcgcta tcaaattatt 780 acattettaa ttgegggaca egtaacaaca agtggtettt tateatttge getgtattte 840 ttagtgaaaa atccacatgt attacaaaaa gcagcagaag aagcagcacg agttctagta 900 gatcctgttc caagctacaa acaagtcaaa cagcttaaat atgtcggcat ggtcttaaac gaagegetge gettatggee aactgeteet gegtttteee tatatgeaaa agaagataeg gtgcttggag gagaatatcc tttagaaaaa ggcgacgaac taatggttct gattcctcag cttcaccgtg ataaaacaat ttggggagac gatgtggaag agttccgtcc agagcgtttt 1140 qaaaatccaa qtqcqattcc qcaqcatqcq tttaaaccqt ttqqaaacqq tcaqcqtqcq 1200 tgtateggte ageagttege tetteatgaa geaaegetgg taettggtat gatgetaaaa 1260 cactttgact ttgaagatca tacaaactac gagctcgata ttaaagaaac tttaacgtta aaacctgaag gctttgtggt aaaagcaaaa tcgaaaaaaa ttccgcttgg cggtattcct 1380 tcacctagca ctgaacagtc tgctaaaaaa gtacgcaaaa aggtagaaaa cgctcataat 1440 acqccqctqc ttqtqctata cqqttcaaat atqqqaacaq ctqaaqqaac qqcqcqtqat 1500 ttagcagata ttgcaatgag caaaggattt gcaccgcagg tcgcaacgct tgattcacac 1560 gccggaaatc ttccgcgcga aggagctgta ttaattgtaa cggcgtctta taacggtcat

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ctaaaagaac aaggacagtc	acttggagaa gcacattta	t actteggetg cegtteacet	2820
catgaagact atctgtatca	agaagagett gaaaaegee	c aaaacgaagg catcattacg	2880
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cgatacgcaa aagacgtgtg	ggctgggtaa		3150

1. A composition for preparing 2-hydroxylated product or 4-hydroxylated product from atorvastatin comprising at least one enzyme selected from a group consisting of wild-type CYP102A1. CYP102A1 mutants, and chimeras derived from the CYP102A1 mutants,

wherein the CYP102A1 mutant has an amino acid sequence changed from that of the wild-type CYP102A1 by at least one substitution selected from a group consisting of substituting arginine (R) at the amino acid position 47 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting tyrosine (Y) at the amino acid position 51 with an amino acid selected from a group consisting of alanine, valine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting glutamic acid (E) at the amino acid position 64 with an amino acid selected from a group consisting of glycine, serine, threonine,

cysteine, tyrosine, asparagine, and glutamine, substituting alanine (A) at the amino acid position 74 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, substituting phenylalanine (F) at the amino acid position 81 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, and tryptophan, substituting leucine (L) at the amino acid position 86 with an amino acid selected from a group consisting of alanine, valine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting phenylalanine (F) at amino acid position 87 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, and tryptophan, substituting glutamic acid (E) at the amino acid position 143 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine,

substituting leucine (L) at the amino acid position 188 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, and substituting glutamic acid (E) at the amino acid position 267 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, and tryptophan, and

the chimera derived from the CYP102A1 mutant has an amino acid sequence changed from that of the reductase domain of the CYP102A1 mutant by at least one substitution selected from a group of substituting lysine (K) at the amino acid position 474 with threonine (T), substituting alanine (A) at the amino acid position 475 with valine (V), substituting glutamine (Q) at the amino acid position 513 with arginine (R), substituting arginine (R) at the amino acid position 526 with proline (P), substituting glutamine (Q) at the amino acid position 547 with glutamic acid (E), substituting glutamic acid (E) at the amino acid position 559 with aspartic acid (D), substituting leucine (L) at the amino acid position 590 with phenylalanine (F), substituting alanine (A) at the amino acid position 591 with serine (S), substituting aspartic acid (D) at the amino acid position 600 with glutamic acid (E), substituting valine (V) at the amino acid position 625 with leucine (L), substituting aspartic acid (D) at the amino acid position 632 with asparagine (N), substituting aspartic acid (D) at the amino acid position 638 with glutamic acid (E), substituting lysine (K) at the amino acid position 640 with alanine (A), substituting alanine (A) at the amino acid position 652 with serine (S), substituting glycine (G) at the amino acid position 661 with arginine (R), substituting threonine (T) at the amino acid position 665 with alanine (A), substituting glutamine (Q) at the amino acid position 675 with lysine (K), substituting proline (P) at the amino acid position 676 with leucine (L), substituting alanine (A) at the amino acid position 679 with glutamic acid, substituting glutamic acid (E) at the amino acid position 688 with alanine (A), substituting threonine (T) at the amino acid position 716 with alanine (A), substituting alanine (A) at the amino acid position 717 with threonine (T), substituting alanine (A) at the amino acid position 742 with glycine (G), substituting alanine (A) at the amino acid position 783 with valine (V), substituting alanine (A) at the amino acid position 796 with threonine (T), substituting lysine (K) at the amino acid position 814 with glutamic acid (E), substituting isoleucine (I) at the amino acid position 825 with methionine (M), substituting arginine (R) at the amino acid position 826 with serine (S), substituting arginine (R) at the amino acid position 837 with histidine (H), substituting glutamic acid (E) at the amino acid position 871 with asparagine (N), substituting isoleucine (I) at the amino acid position 882 with valine (V), substituting glutamic acid (E) at the amino acid position 888 with glycine (G), substituting aspartic acid (D) at the amino acid position 894 with glycine (G), substituting proline (P) at the amino acid position 895 with serine (S), substituting glycine (G) at the amino acid position 913 with serine (S), substituting glutamic acid (E) at the amino acid position 948 with lysine (K), substituting serine (S) at the amino acid position 955 with asparagine (N), substituting methionine (M) at the amino acid position 968 with valine (V),

- substituting glutamine (Q) at the amino acid position 971 with glutamic acid (E), substituting methionine (M) at the amino acid position 980 with valine (V), substituting glutamine (Q) at the amino acid position 982 with arginine (R), substituting alanine (A) at the amino acid position 1009 with aspartic acid (D), substituting aspartic acid (D) at the amino acid position 1020 with glutamic acid (E), substituting histidine (H) at the amino acid position 1022 with tyrosine (Y), substituting glutamine (Q) at the amino acid position 1023 with lysine (K) and glutamic acid (E), and substituting glycine (G) at the amino acid position 1040 with serine (S).
- 2. The composition of claim 1, wherein the CYP102A1 mutant has an amino acid sequence changed from that of the wild-type CYP102A1 by at least one substitution selected from a group consisting of substituting arginine (R) at the amino acid position 47 with leucine (L), substituting tyrosine (Y) at the amino acid position 51 with phenylalanine (F), substituting glutamic acid (E) at the amino acid position 64 with glycine (G), substituting alanine (A) at the amino acid position 74 with glycine (G), substituting phenylalanine (F) at the amino acid position 81 with isoleucine (I), substituting leucine (L) at the amino acid position 86 with isoleucine (I), substituting phenylalanine (F) at the amino acid position 87 with valine (V), substituting glutamic acid (E) at the amino acid position 143 with glycine (G), substituting leucine (L) at the amino acid position 188 with glutamine (Q), and substituting glutamic acid (E) at the amino acid position 267 with valine (V).
- 3. The composition of claim 1, wherein the CYP102A1 mutant includes an amino acid substitution position and substituted amino acid in the wild-type CYP102A1 selected from a group consisting of F87A,

R47L/Y51F, A74G/F87V/L188Q, R47L/L86I/L188Q, R47L/F87V/L188Q, R47L/F87V/L188Q/E267V, R47L/L86I/L188Q/E267V, R47L/L86I/L188Q/E267V, R47L/E86I/F87V/L188Q, R471F87V/E143G/L188Q/E267V, R47L/E64G/F87V/E143G/L188Q/E267V, R47L/F811I/F87V/E143G/L188Q/E267V, and R47L/E64G/F81I/F87V/E143G/L188Q/E267V.

- **4.** The composition of claim **1**, wherein the chimera derived from the CYP102A1 mutant includes an amino acid substitution position and substituted amino acid in the CYP102A1 mutant selected from a group consisting of
 - A475V/E559D/T665A/P676L/A679E/E688A/A742G/ K814E/R826S/R837H/E871N/I882V/E888G/P895S/ S955N/M968V/Q982R/A1009D/H1022Y/Q1023E,
 - A475V/E559D/T665A/A679E/E688A/A742G/K814E/ E87 N/I882V/E888G/P895S/G913 G/S955N/M968V/ A1009D/H1022Y/Q1023E, K474T/A475V/A591S/ D600E/V625L/D632N/K640A/T665A/A717T/ A742G/A796T/K814E/I825M/I882V/E888/S955N/ M968V/M980V/A1009D/D1020E/Q1023K/G1040S,
 - K474T/A475V/R526P/Q547E/D600E/V625L/D638E/ K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K 814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/Q971E/A1009D/ D1020E,
 - K474T/A475V/Q513R/Q547E/D600E/V625L/D638E/ K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/A 009D/D1020E, K474T/A475V/Q547E/D600E/V625L/D638E/K640A/

G661R/T665A/Q675K/T716A/A717T/A742G/

A783V/K814E/I825M/E871N/I882V/E888G/D894G/ E948K/S955N/M968V/A1009D/D1020E, and K474T/A475V/Q547E/L590F/D600E/V625L/D638E/ K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/A1009D/D1020E.

5. A method for preparing 2-hydroxylated product or 4-hydroxylated product from atorvastatin comprising reacting at least one enzyme selected from a group consisting of wild-type CYP102A1, CYP102A1 mutants, and chimeras derived from the CYP102A1 mutants with atorvastatin,

wherein the CYP102A1 mutant has an amino acid sequence changed from that of the wild-type CYP102A1 by at least one substitution selected from a group consisting of substituting arginine (R) at the amino acid position 47 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting tyrosine (Y) at the amino acid position 51 with an amino acid selected from a group consisting of alanine, valine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting glutamic acid (E) at the amino acid position 64 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, substituting alanine (A) at the amino acid position 74 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, substituting phenylalanine (F) at the amino acid position 81 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, and tryptophan, substituting leucine (L) at the amino acid position 86 with an amino acid selected from a group consisting of alanine, valine, isoleucine, proline, methionine, phenylalanine, and tryptophan, substituting phenylalanine (F) at amino acid position 87 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, and tryptophan, substituting glutamic acid (E) at the amino acid position 143 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, substituting leucine (L) at the amino acid position 188 with an amino acid selected from a group consisting of glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine, and substituting glutamic acid (E) at the amino acid position 267 with an amino acid selected from a group consisting of alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, and tryptophan, and

the chimera derived from the CYP102A1 mutant has an amino acid sequence changed from that of the reductase domain of the CYP102A1 mutant by at least one substitution selected from a group of substituting lysine (K) at the amino acid position 474 with threonine (T) substituting alanine (A) at the amino acid position 475 with valine (V), substituting glutamine (Q) at the amino acid position 513 with arginine (R), substituting arginine (R) at the amino acid position 526 with proline (P), substituting glutamine (Q) at the amino acid position 547 with glutamic acid (E), substituting glutamic acid (E) at the amino acid position 559 with aspartic acid (D), substituting leucine (L) at the amino acid position 590 with phenylalanine (F), substituting alanine (A) at the amino

acid position 591 with serine (S), substituting aspartic acid (D) at the amino acid position 600 with glutamic acid (E), substituting valine (V) at the amino acid position 625 with leucine (L), substituting aspartic acid (D) at the amino acid position 632 with asparagine (N), substituting aspartic acid (D) at the amino acid position 638 with glutamic acid (E), substituting lysine (K) at the amino acid position 640 with alanine (A), substituting alanine (A) at the amino acid position 652 with serine (S), substituting glycine (G) at the amino acid position 661 with arginine (R), substituting threonine (T) at the amino acid position 665 with alanine (A), substituting glutamine (Q) at the amino acid position 675 with lysine (K), substituting proline (P) at the amino acid position 676 with leucine (L), substituting alanine (A) at the amino acid position 679 with glutamic acid, substituting glutamic acid (E) at the amino acid position 688 with alanine (A), substituting threonine (T) at the amino acid position 716 with alanine (A), substituting alanine (A) at the amino acid position 717 with threonine (T), substituting alanine (A) at the amino acid position 742 with glycine (G), substituting alanine (A) at the amino acid position 783 with valine (V), substituting alanine (A) at the amino acid position 796 with threonine (T), substituting lysine (K) at the amino acid position 814 with glutamic acid (E), substituting isoleucine (I) at the amino acid position 825 with methionine (M), substituting arginine (R) at the amino acid position 826 with serine (S), substituting arginine (R) at the amino acid position 837 with histidine (H), substituting glutamic acid (E) at the amino acid position 871 with asparagine (N), substituting isoleucine (I) at the amino acid position 882 with valine (V), substituting glutamic acid (E) at the amino acid position 888 with glycine (G), substituting aspartic acid (D) at the amino acid position 894 with glycine (0), substituting proline (P) at the amino acid position 895 with serine (S), substituting glycine (G) at the amino acid position 913 with serine (S), substituting glutamic acid (E) at the amino acid position 948 with lysine (K), substituting serine (S) at the amino acid position 955 with asparagine (N), substituting methionine (M) at the amino acid position 968 with valine (V), substituting glutamine (O) at the amino acid position 971 with glutamic acid (E), substituting methionine (M) at the amino acid position 980 with valine (V), substituting glutamine (Q) at the amino acid position 982 with arginine (R), substituting alanine (A) at the amino acid position 1009 with aspartic acid (D), substituting aspartic acid (D) at the amino acid position 1020 with glutamic acid (E), substituting histidine (H) at the amino acid position 1022 with tyrosine (Y), substituting glutamine (Q) at the amino acid position 1023 with lysine (K) and glutamic acid (E), and substituting glycine (G) at the amino acid position 1040 with serine (S).

- **6**. The method of claim **5**, further comprising adding a NADPH-generating system.
- 7. The method of claim 6, wherein the NADPH-generating system includes glucose 6-phosphate, NADP+, and yeast glucose 6-phosphate dehydrogenase.
- 8. The method of claim 5, wherein the CYP102A1 mutant has an amino acid sequence changed from that of the wild-type CYP102A1 by at least one substitution selected from a group consisting of: substituting arginine (R) at the amino acid position 47 with leucine (L), substituting tyrosine (Y) at

the amino acid position 51 with phenylalanine (F), substituting glutamic acid (E) at the amino acid position 64 with glycine (G), substituting alanine (A) at the amino acid position 74 with glycine (G), substituting phenylalanine (F) at the amino acid position 81 with isoleucine (I), substituting leucine (L) at the amino acid position 86 with isoleucine (I), substituting phenylalanine (F) at the amino acid position 87 with valine (V), substituting glutamic acid (E) at the amino acid position 143 with glycine (G), substituting leucine (L) at the amino acid position 188 with glutamine (Q), and substituting glutamic acid (E) at the amino acid position 267 with valine (V).

9. The method of claim 5, wherein the CYP102A1 mutant includes an amino acid substitution position and substituted amino acid in the wild-type CYP102A1 selected from a group consisting of F87A.

R47L/Y51F, A74G/F87V/L188Q, R47L/L86I/L188Q, R47L/F87V/L188Q, R47L/F87V/L188Q, R47L/F87V/L188Q/E267V, R47L/L86I/L188Q/E267V, R47L/L86I/L188Q/E267V, R47L/E64G/F87V/E143G/L188Q/E267V, R47L/F811/F87V/E143G/L188Q/E267V, and R47L/E64G/F811/F87V/E143G/L188Q/E267V.

- 10. The method of claim 5, wherein the chimera derived from the CYP102A1 mutant includes an amino acid substitution position and substituted amino acid in the CYP102A1 mutant selected from a group consisting of
 - A475V/E559D/T665A/P676L/A679E/E688A/A742G/ K814E/R826S/R837H/E871N/I882V/E888G/P895S/ S955N/M968V/Q982R/A1009D/H1022Y/Q1023E,
 - A475V/E559D/T665A/A679E/E688A/A742G/K814E/ E871N/I882V/E88G/P895S/G913G/S955N/M968V/ A1009D/H1022Y/Q1023E,
 - K474T/A475V/A591S/D600E/V625L/D632N/K640A/ T665A/A717T/A742G/A796T/K814E/I825 M/I882V/ E888/S955N/M968V/M980V/A1009 D/D1020E/ O1023K/G1040S.
 - K474T/A475V/R526P/Q547E/D600E/V625L/D638E/ K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E8880/ D894G/E948K/S955N/M968V/Q971E/A1009D/ D1020E,
 - K474T/A475V/Q547E/D600E/V625L/D638E/K640A/ G661R/T665A/Q675K/T716A/A717T/A742G/ A783V/K814E/I825M/E871N/I888G/D894G/E948K/ S955N/M968V/A1009D/D1020E,
 - K474T/A475V/Q547E/D600E/V625L/D638E/K640A/ G661R/T665A/Q675K/T716A/A717T/A742G/ A783V/K814E/I825M/E871N/I882V/E888G/D894G/ E948K/S955N/M968V/A1009D/D1020E, and
 - K474T/A475V/Q547E/L590F/D600E/V625L/D638/ K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/A1009D/D1020E.

- 11. A kit for preparing 2-hydroxylated product or 4-hydroxylated product from atorvastatin comprising at least one enzyme selected from a group consisting of wild-type CYP102A1, CYP102A1 mutants, and chimeras derived from the CYP102A1 mutants and the NADPH-f-generating system.
 - wherein the CYP102A1 mutant includes an amino acid substitution position and substituted amino acid in the wild-type CYP102A1 selected from a group consisting of F87A, R47L/Y51F,
 - A74G/F87V/188Q, R47L/L86I/L188Q, R47L/F87V/ 188Q,
 - R47L/F87V/L188Q/E267V, R47L/L86I/L188Q/E267V, R47L/L86I/F87V/L188Q, R47L/F87V/E143G/L188Q/E267V.

R47L/E64G/F87V/E143G/L188Q/E267V,

R47L/F81I/F87V/E143G/L188Q/E267V, and

R47L/E64G/F81I/F87V/E143G/L188Q/E267V, and

- the chimera derived from the CYP102A1 mutant includes an amino acid substitution position and substituted amino acid in the CYP102A1 mutant selected from a group consisting of
- A475V/E559D/T665A/P676L/A679E/E688A/A742G/ K814E/R826S/R837H/E871N/I882V/E888G/P895S/ S955N/M968V/Q982R/A1009D/H1022Y/Q1023E,
- A475V/E559D/T665A/A679E/E688A/A742G/K814E/ E871N/I882V/E888G/P895S/G913G/S955N/M968V/ A1009D/H1022Y/Q1023E,
- K474T/A475V/A591S/D600E/V625L/D632N/K640A/ T665A/A717T/A742G/A796T/K814E/I825M/I882V/ E888/S955N/M968V/M980V/A1009D/D1020E/ Q1023K/G1040S,
- K474T/A475V/R526P/Q547E/D600E/V625 L/D638E/ K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/Q971E/A1009D/ D1020E,
- K474T/A475V/Q513R/Q547E/D600E/V625L/D638E/ K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/A1009D/D1020E,
- K474T/A475V/Q547E/D600E/V625L/D638E/K640A/G661R/T665A/Q675K/T716A/A717T/A742G/A783V/K814E/I825M/E871N/I882V/E888G/D894G/E948K/S955N/M968V/A1009D/D1020E, and
- K474T/A475V/Q547E/L590F/D600E/V625L/D638E/ K640A/G661R/T665A/Q675K/T716A/A717T/ A742G/A783V/K814E/I825M/E871N/I882V/E888G/ D894G/E948K/S955N/M968V/A1009D/D1020E.
- 12. The kit of claim 11, wherein the NADPH-generating system includes glucose 6-phosphate. NADP+, and yeast glucose 6-phosphate dehydrogenase.

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