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Computational comparison of β-mannosidases of animals, humans, microbes, and plants

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Abstract: The β-mannosidase (MANB) enzyme is involved in removing mannose residue from the nonreducing end, and its impaired activity leads to β-mannosidosis. MANB amino acid sequences of humans, other mammals, plants, fungi, and bacteria were compared to determine their similarities, differences, and predicted 3D structures. Our cloned MANB DNA sequence showed a 99% similarity to a previously reported human MANB DNA sequence but 16 nucleotide differences were observed, showing the polymorphic nature of the enzyme. The 9 changed codons coded the different amino acids *Ile*, *Lys*, *Ile*, *Thr*, *Arg*, *Leu*, *Leu*, *Gly*, and *Asp*, while 7 changed codons coded the same amino acids, *Ile*, *Arg*, *Gln*, *Val*, *Ile*, *Pro*, and *Val*. The amino acid sequence comparison of human MANB with bovine, goat, and mouse MANB showed a nearly 75% similarity, while 10%-13%, 17%-18%, and 9%-23% similarities were observed with plant, fungi, and bacteria MANB, respectively. The catalytic nucleophilic and proton donor sites were conserved in the β-mannosidase of mammals, plants, fungi, and bacteria, except *L. esculentum*, and the nucleophilic site of *P. furiosus* was also changed. The catalytic sites of MANB indicated that it follows a dyad catalytic mechanism. Additionally, 2 common putative glycosylation sites at N-residues 35 and 77 were conserved. The 3D structure prediction indicated differences in the α-helix loop, while the β-pleated sheets were nearly the same. The comparison showed that the MANB enzyme is polymorphic in nature with conserved catalytic sites and has an evolutionary relationship among different species. The 3D structure comparison of MANB will be helpful to understand the disease process of β-mannosidosis.

Key words: β-Mannosidase, DNA sequence, amino acid comparison, computational analysis

Introduction

β-Mannosidase (MANB, β-D-mannosidase mannohydrolase, EC 3.2.1.25) is one of the exoglycosidases that cleave the single β-linked mannose residue from the nonreducing end of all N-linked glycoproteins. It is essential for the complete hydrolysis of polysaccharides such as galactoglucomannan and mannooligosaccharides, which are produced by β-mannanase activity. MANB is also important in the saccharification of hemicelluloses to monomeric sugar for further conversion into biochemical products as well as in the synthesis of oligosaccharides. Oligosaccharides are long chains of sugar molecules used in the building of bones, cartilage, skin, tendons, and many other tissues in the body (1-4). This enzyme also provides useful information for structural studies of polysaccharides and glycoproteins that have β-mannosidic linkages.

MANB enzyme has been purified from fungi (1,5), hyperthermophilic microbes (6,7), plants (8,9), goats (10), bovines (3), and humans (11). The defective

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or impaired activity of MANB leads to a lysosomal storage disease commonly known as β-mannosidosis. This disease has been detected in Nubian goats (12) and bovines (2,13), as well as in humans (14,15). Facial dysmorphism, abnormal skull and joints, weakness and shrinkage of muscles, marked intention tremor, and deafness have been reported as pathological symptoms (16). The defective activity of the MANB enzyme leads to the accumulation of mannose-conjugated oligosaccharides or glycoproteins in many tissues, along with the loss of the myelin sheath in the nervous system (17).

In humans, the clinical expressions of β-mannosidosis are reported as peripheral neuropathy (18,19), hearing loss, speech impairment (20), epileptic encephalopathy (21), and angiokeratoma (22). The MANB enzyme activity was completely lost in the fibroblast, plasma, and leukocytes of β-mannosidosis patients (16).

As a continuation of our previous report about the cloning and expression of the MANB gene, there is a need for computational analysis of MANB DNA and its amino acid sequence similarity to or differences from reported MANB DNA and amino acid sequences of other species. The computational analysis will be helpful in finding the evolutionary conservation as well as in understanding the degradation pathway of mannose-linked glycoproteins in the progression of β-mannosidosis in mammals.

Materials and methods

Comparison of DNA sequences of human β-mannosidase

The cloned DNA sequence of human β-mannosidase (EU009130) was compared with the reported human β-mannosidase DNA sequence (U60337) (15). Both DNA sequences were aligned using the nucleotide-nucleotide BLAST (BLASTN) program of the National Center for Biotechnology Information (NCBI), and the nucleotide differences were noted.

Comparison of deduced amino acid sequences of β-mannosidase

MANB enzyme activity was characterized in the species mentioned below and their NCBI accession numbers were used to learn the deduced amino acid sequences of MANB from the NCBI. The deduced amino acid sequences of MANB of different species were aligned with deduced amino acid sequences of our cloned human MANB in ClustalW 1.83 and similarities and differences of the amino acid residues were noted.

1) Mammals: human (*Homo sapiens*) (U60337), bovine (*Bos taurus*) (U17432), goat (*Capra hircus*) (U46067), and mouse (*Mus musculus*) (AF306557).

2) Fungi: *Aspergillus niger* (AJ251874) and *Aspergillus aculeatus* (AB015509).

3) Plants: Arabidopsis (*Arabidopsis thaliana*) (AB122060), cotton (*Gossypium hirsutum*) (AY187062), and tomato (*Lycopersicon esculentum* Mill.) (AF415204).

4) Bacteria: *Cellulomonas fimi* (AF126472), *Thermotoga neapolitana* (AY033395), *Thermobifida fusca* (CAD33708), *Pyrococcus furiosus* (U60214), *Streptomyces coelicolor* (NP_630333), *Xylella fastidiosa* (NP_298136), and *Thermotoga maritime* (NP_229424).

Prediction of 3-dimensional structures

The MANB amino acid sequences of human, bovine, goat, mouse, *Cellulomonas fimi*, *Thermotoga neapolitana*, *Pyrococcus furiosus*, *Streptomyces coelicolor*, and *Thermotoga maritime* were inserted into the ExPASy proteomics server and the 3-dimensional structures were predicted.

Results

Sequence comparison

Human

The cloned human MANB DNA sequence was aligned with the reported human MANB cDNA sequence. A 99% DNA sequence similarity was observed, but 16 nucleotide differences in the open reading frame of β-mannosidase DNA were identified. The positions of nucleotide differences are shown in the Table. Due to a change in triplet codons, it was noted that 7 triplet codons with changed nucleotide positions at 390, 479, 486, 489, 757, 1011, and 1137 maintained the same amino acid residues as *Ile*, *Arg*, *Gln*, *Val*, *Ile*, *Pro*, and *Val*, respectively. In

Table. Computational comparison of DNA sequences of the human β-mannosidase gene and deduced amino acid sequences.

No.	Nucleotide position	Alkhayat (U60337)	Amino acid residue triplet codon	Samra (EU009130)	Amino acid residue triplet codon
1.	208	**C**AC	His	T**A**C	Tyr
2.	390	AT**T**	Ile*	A**T**C	Ile*
3.	479	CSC	Arg*	C**G**C	Arg*
4.	486	CA**G**	Gln*	CA**A**	Gln*
5.	489	GT**T**	Val*	GT**C**	Val*
6.	757	**N**TA	Val/Ile*	**A**TA	Ile*
7.	862	**G**AA	Glu	**A**AA	Lys
8.	889	A**A**C	Asn	A**T**C	Ile
9.	1011	CC**T**	Pro*	CC**C**	Pro*
10.	1037	A**A**T	Asn	A**C**T	Thr
11.	1039	**G**GA	Gly	**C**GA	Arg
12.	1130	**C**AG	Gln	**C**TG	Leu
13.	1137	GT**T**	Val*	GT**A**	Val*
14.	1598	**T**TT	Phe	**C**TT	Leu
15.	1766	G**A**A	Glu	G**G**A	Gly
16.	2590	**A**AT	Asn	**G**AT	Asp

Bold letters indicate nucleotide differences. Asterisk indicates common amino acid residue.

the 9 remaining triplet codons, the first nucleotide position of the triplet codons was changed at 208, 862, 1039, 1130, 1598, and 2590, while the second nucleotide position was changed at 889, 1037, and 1766 (Table). The positions of the changed amino acids numbers are shown (Figure 1).

Other mammals

The comparison of deduced amino acid sequences of human β-mannosidase with MANB of mouse, bovine, and goat indicated that a 74.85% sequence similarity was observed with bovine and mouse MANB, whereas a 74.63% similarity was observed with goat MANB. The proposed catalytic nucleophilic site (Glu-554) and proton donor site (Glu-457) of human β-mannosidase was suggested after alignment with an amino acid sequence of *A. thaliana* β-mannosidase (9). The common potential glycosylation sites of MANB were observed at N-residues 35 and 57 of goat, human, and bovine, but not in mouse, while glycosylation at residues 28, 280, 284, and 763 was only observed in human MANB. The glycosylation at residue 297 is conserved among bovine, mouse, human, and goat.

Plant

The alignment of deduced amino acids of human β-mannosidase with reported amino acids sequences of MANB of *G. hirsutum* and *A. thaliana* showed a 13.42% and a 13.69% similarity, respectively, while it showed a 10.70% similarity with tomato β-mannosidase. The catalytic nucleophilic and proton donor sites of β-mannosidase are conserved in human, cotton, and Arabidopsis, and was not found conserved in *L. esculentum* β-mannosidase (Figure 2).

```
        1        253    288   297  346  347  377  533  589   864     879
Samra   Met------I------K-----I----T----R----L----L----G-----D-----stop
          ------ ------ ----- ---- ---- ---- ---- ---- ----- -----
Alkhyat Met-----V/I-----E-----N----N----G----Q----F----E-----N-----stop
```

Figure 1. Alignment and comparison of deduced amino acid sequences of human β-mannosidase (EU009130) and reported human β-mannosidase (Alkhayat, U60337). The amino acids differences are represented by numbers with 1-letter amino acid abbreviations. Dashes indicate the same amino acid sequence. The 9 changed amino acid residues are shown.

```
Amino acid    1  48  55 136  184 185 236 282 295 297 299 328 380 390
residue no    1  48  55 134  181 182 233 279 292 294 296 325 377 387
of species    1  45   5 119  178 179 224 266 280 282 284 312 369 379
              1   6  13  54   62  63  79 100 114 116 118 130 173 183

G.hirsutum    M--P--L---T---W----D---E---F---P--G---G---R----D---G
A.thaliana    M--P--L---T---W----D---E---F---P--G---G---R----D---G
SMANB         M--P--L---T---W----D---E---F---P--G---G---R----D---G
L.esculentum  M--P--L---T---W----D---E---F---P--G---G---R----D---G
              *   *   *   *    *   *   *   *   *  *   *   *    *   *
```

```
Amino acid    399 414 453 455 465 558 597 623 909 933    976
residue no    396 411 450 452 464 549 588 612 876 897    944
of species    388 403 434 435 457 554 578 592 803 827    879
              192 207 240 242         334 349 452 476    514

G.hirsutum     F---F---H---S---E---E---N---P---F---D------L
A.thaliana     F---F---H---S---E---E---N---P---F---D------G
SMANB          F---F---H---S---E---E---N---P---F---D------Y
L.esculentum   F---F---H---S-----------N---P---F---D------H
              *   *   *   *   ▲   ▲   *   *   *   *
```

Figure 2. Computational comparison of deduced amino acid sequences of human and plant β-mannosidases. SMANB represents cloned human β-mannosidase (EU009130). Amino acid sequence alignment of human with reported plant β-mannosidase of *A. thaliana* (AB122060), *G. hirsutum* (AY187062), and *L. esculentum* (AF415204) was done using ClustalW 1.83. The common conserved aligned amino acid sequence is shown by an asterisk. The proposed catalytic nucleophilic (*Glu-549*) and proton donor (Glu-*464*) sites of plant endo-β-mannosidase of *A. thaliana* (9) are marked with a bolded "**E**" and shown by an arrowhead. These sites were used to compare the catalytic sites of other β-mannosidases. Dashes indicate the nonconserved amino acid sequences.

Fungi

The comparison of the deduced amino acid sequence of human MANB with the reported amino acid sequences of β-mannosidase of *A. niger* and *A. aculeatus* showed 18.99% and 17.86% similarity, respectively (Figure 3). The first amino acid after the start codon "M" is "R", which resembles the amino acid sequence of human β-mannosidase and suggests the pattern of the eukaryotic translation signal sequence (23). One glycosylation site was also found conserved in the β-mannosidase of *A. niger*, *A. aculeatus*, and human. The proposed catalytic nucleophilic and proton donor sites are also conserved.

Bacteria

The deduced amino acid sequences of bacterial β-mannosidases showed conserved proton donor and

```
A.niger     MRHSIGLAAALLAPTLPVALG--QHIRDLSSEKWTLSSRALNRTVPAQFPSQVHLDLLRA 58
A.aculaetus MRALPTTATTLLGVLFFPSASRSQYVRDLGTEQWTLSSATLNRTVPAQFPSQVHMDLLRE 60
SMANB       MRLHLLLLLLALCGAGTTAAELS-----YSLRGNWSICNGNGSLELPGAVPGCVHSALFQQ 55
            **       :* .     :             :*::..   .  :*. .*. **  *::

A.niger     GVIGEYHG-LNDFNLRWIAAANWTYT-SQPIKGLLDNYGSTWLVFDGLDTFATISILWTA 58
A.aculaetus GIIDEPYNDLNDFNLRWIADANWTYT-SGKIEGLGEDYESTWLVFDGLDTFASISFCGQF 59
SMANB       GLIQDSYYRFNDLNYRWVSLDNWTYSKEFKIPFEISKWQKVNLILEGVDTVSKILFNEVT 60
            *:* : :  :**:* **::  ****: .  *    ..: .. *:::*:**.:.* :

A.niger     NRIHGQSVSPVSGSMYLPALEAC-QRRILIRKVSFRGGVTAEVNTCYLHIEWPDDVQLTY 59
A.aculaetus VGATDNQFRQYMFDVSSILKACP-EEPTLGIQFGSAPNIVDAIAQDPSSPTWPEGVQITY 59
SMANB       IGETDNMFNRYSFDITNVVRDVNSIELRFQSAVLYAAQQSKAHTRYQVPPDCPPLVQKGE 60
                .: .     .:          .  :   .                    *  **

A.niger     EYPNRWFMRKEQSDFGWDWGPAFAPAGPWKPAYIVQLDKKESVYVLNTDLDIYRKNQINY 60
A.aculaetus EYPNRWFMRKEQSDFGWDWGPAFAPAGPWKPGYVVQLKQAAPVYVRNTDLDIYRLGQINY 60
SMANB       CHVN--FVRKEQCSFSWDWGPSFPTQGIWKDVRIEAY---------NICHLNYFTFSPIY 49
             : *  *:****..*.*****:*.. * **   :           *     *   .  *

A.niger     LPPDQSQPWVVNASIDILGPLPAKPTMSIEVRDTHSGTILTSRTLNNVSVAGNAITGVTV 60
A.aculaetus LPPDQTQPWVVNASLDYLGSLPENPSMAIEVKDLQSGEILASRPLTNITVTEGSVTGVTV 60
SMANB       DKSAQEWNLEIESTFDVVSSKPVGGQVIIAIPKLQTQQTYSIELQPGKRIVELFVN---I 57
              . *     :::::* :.. *    : * : . ::    : .   .  :.   :.   :

A.niger     LDGLNPKLWWPQSSVIRTSTMFLSLSKVEGTRPWPVWTNGRASAPFFLNQRNITEVQRAQ 60
A.aculaetus LEGVDPKLWWPQGLGDQNLYNVTISVTDGGNQSVAEVTKRTGFRTIFLNQRNITDAQLAQ 60
SMANB       SKNITVKTWWPHGHGIQTGYNMTVLFELDG--------GLNIEKSAKVYFRTVELIEEPI 52
             ..:  * ***:.   :.   .       *             .  :  *.:   : .

A.niger     GIAPGANWHFEVNGHEFYAKGSNLIPPDSFWTRVTEERISRLFDAVVVGNQNMLRVWSSG 60
A.aculaetus GIAPGANWHFEVNGHEFYAKGSNLIPPDCFWTRVTEDTMTRLFDAVVAGNQNMLRVWSSG 60
SMANB       KGSPGLSFYFKITRFPIFLKGSNWIPADSFQDRVTSELLRLLLLSVVDANMNTLRVWGGG 60
              :** .::*::. . :: **** **.*.*  ***.: :  *: :** .* * ****..*

A.niger     AYLHDYIYDLADEKGILLWSEFEFSDALYPSDDAFLENVAAEIVYNVRRVNHHPSLALWA 60
A.aculaetus AYLHDYIYDLADEKGILLCSEFQFSDALYPTDDAFLENVAAEVVYNVRRVNHHPSLALWA 60
SMANB       IYEQDEFYELCDELGIMVWQDFMFACALYPTDQGFLDSVTAEVAYQIKRLKSHPSIIIWS 60
             * :* :*:*.** **:: .:* *: ****:*:.**:.*:**:.*:::*:: ***: :*:
               ▼
A.niger     GGNEIESLMLPRVKDAAPSSYSYYVGEYEKMYISLFLPLVYENTRSISYSPSSTTEGYLY 60
A.aculaetus GGNEIESLMLLLVEAADPESYPFYVGEYEKMYISLFLPLVYENTRSISYSPSSTTEGYLD 60
SMANB       GNNENEEALMMNWYHISFTDRPIYIKDYVTLYVKNIRELVLAGDKSRPFITSSPTNG--- 57
            *.** *. ::         . . *: :* .:*:. :  **  . :* .: .**.*:*
                                                        ▼
A.niger     IDLSAPVPMAERYDNTTSGSYYGDTDHYDYDTSVAFDYGSYPVGRFANEFGFHSMPSLQT 60
A.aculaetus IDLSAPVPMAERYSNTTEGEYYGDTDHYNYDASIAFDYGTYPVGRFANEFGFHSMPSLQT 60
SMANB       ----AETVAEAWVSQNPNSNYFGDVHLYDYIS-DCWNWKVFPKARFASEYGYQSWPSFST 56
                * .      .:.....*:**.. *:* :  .:::  :* .***.*:*::* **:.*

A.niger     WQQAVDT-EDLYFNSSVVMLRNHHDPAGGLMTDNYANSATGMGEMTMGVISYYPIPSKSD 59
A.aculaetus WQQALTDPADLTFNSSVVMLRNHHYPAGGLMTDNYHNTVARHGRNDPGRAGLLPDAQHSV 60
SMANB       LEKVSST-EDWSFNSKFSLHRQHHGGGN---------------KQMLYQAGLHFKLPQST 44
             ::.     *  ***.. : *:**  ..               .      .      :*

A.niger     HISN-FSAWCHATQLFQADMYKSQIQFYRRGSGMPERQ----LGSLYWQLEDIWQAPSWA 55
A.aculaetus RPRGQLQRLVPRDPALPGGPLQVTNPVLPAGQRAARTP----ARVPVLAARGHLAGALVG 56
SMANB       DPLRTFKDTIYLTQVMQAQCVKTETEFYRRSRSEIVDQQGHTMGALYWQLNDIWQAPSWA 60
                 :.       : .   :    .   .                    ..   ..  .
```

Figure 3.

```
A.niger     GIEYGGRWKVLHHVMRDIYQPVIVSPFWN-YTTGSLDVYVTSDLWSPAAGTVDLTWLDLS 59
A.aculaetus GDRVRRPLEGPHYVARDIYKPVIVSPFWN-YTTGALDIYVTSDLWTAAAGSVTLTWRDLS 59
SMANB       SLEYGGKWKMLHYFAQNFFAPLLPVGFENENTFYIYGVSDLHSDYSMTLSVRVHTWSSLE 60
            . .     :  *:. :::: *::   * *  *    .:    . :: : .    ** .*.

A.niger     GRPIAGNAGTP-KSVPFTVGGLNSTRIYGTNVSSLGLPDTKDAVLILSLSAHGRLPNSDR 59
A.aculaetus GKPIASNGGLPTKPLPFHVGALNSTRLYRMNMKQQPLPRHEDAILALELTATGSLPNTDE 60
SMANB       PVCSR-------VTERFVMKGGEAVCLYEEPVS--ELLRRCGNCTRESCVVSFYLSADHE 51
                        .  * : . ::. :*   :.   *    .     .  .   *.  ..

A.niger     TTNLTHENYATLSWPKDLKIVDPGLKLGYSSKKTTVTVEATSGVSLYTWLDYPEGVVGYF 60
A.aculaetus EVTFTHEQWFTPAFPKDLDLVNLRVRVEYDAPLGKFAVEATAGVALYTWLEHPEGVVGYF 60
SMANB       LLSPTNYHFLSSPKEAVGLCKAQITAIISQQGDIFVFDLETSAVAPFVWLDVGS-IPGRF 59
              . *: :: : .             :  .     .    *:.*: :.**:  . : * *

A.niger     EENAFVLAPGEKKEIGFTVLDDTTNGAWVRNITVQSLWDQKVRG 43
A.aculaetus EENSFVVVPGQKKVVGFVVQADETDGEWVHDVTVRSLWDLNEGE 43
SMANB       SDNGFLMTEKTR-TILFYPWEPTSKDELEQSFHVTSLTDIY--- 39
              .:*. ::.   :  : :      :..   :.. * :* :
```

Figure 3. Amino acid sequence alignment of human β-mannosidase (SMANB, EU009130) with *A. niger* and *A. aculeatus* was done using ClustalW 1.83. The asterisk indicates a conserved amino acid sequence, (.) indicates conserved substitution, and (:) indicates semiconserved substitution. The proposed catalytic nucleophile (*Glu-554*) and proton donor (*Glu-457*) sites based on the human β-mannosidase are marked with a bold "**E**" and shown by an arrowhead. One common glycosylation site is marked with a bold "**N**."

catalytic nucleophilic sites, except in *P. furiosus*. DNA sequences of β-mannosidase in *L. esculentum* and *P. furiosus* were observed to be shorter as compared to mammalian β-mannosidase. It was also noted that in hyperthermophilic *P. furiosus*, the proton donor site at 320 is comparable with the mammalian proton donor site at amino acid residue 457, but the catalytic nucleophilic site of *P. furiosus* changed and shifted at amino acid residue 395 (Figure 4).

3D structure

The predicted 3-dimensional structures of MANB enzymes were divided into left (L), center (C), and right (R) parts. The comparison of the 3D structures of MANB enzymes of mammals indicated that in part L, 'a' has the same type of β-pleated sheets. In part C, an additional loop of α-helix structures was observed in bovine and goat, while mouse resembles the 3D structure of human MANB enzyme (Figure 5). Part L of the mammals is different from part L of bacteria. Further comparison of the 3D structure of human MANB with the 3D structure of MANB of *C. fimi* and *P. furiosus* indicated an extra extended loop of β-pleated sheets in *C. fimi* and 2 extra β-pleated sheets in *P. furiosus*. The numbers of α-helix turns in part C are also different between mammalian and bacterial MANB enzymes.

Discussion

β-Mannosidase is an important enzyme in the degradation pathway of mannose polymers and mannose-linked glycoproteins. The deduced amino acid sequence of human β-mannosidase was aligned with the β-mannosidases of animals, plants, and microbes. A 75% similarity of deduced amino acid sequence was observed between human and animal β-mannosidases, while a 10%-23% similarity was observed when comparing human β-mannosidase with plant and microbe β-mannosidase. The catalytic nucleophilic and proton donor sites and 2 glycosylation sites are conserved among the β-mannosidases of all species. In the 3D structures, a difference in α-helix structures was observed while the β-pleated sheets were nearly the same.

The β-mannosidases of all species are active at an acidic pH (5.0 to 5.5), except that of *P. furiosus*, which shows enzyme activity at pH levels of 7.2 to 7.3. The glycosylation sites of β-mannosidases are

```
Amino acid      1--29--48—78—120—184—289—423—435—455—457—501--554
residue no      1--10--29—59—110—164—255—384—397—417—419—460--505
                1--10--29—59—110—164—255—381—397—417—419—460--505
                1--21--48—78—128—183—280—409—421—441—443—484--531
                1--11--28—58—114—165—257—393—405—425—427—468--515
                1--17--41—71—120—175—265—396—408—428—430—471--518
                1--38--68—98—149—204—313—442—454-474—476—526--574
                1------10—40--74—105—163—285—297—318—320—361-----

SMANB           M---W---V---W---D---W---P----P---E----N----E---P-----E
T.neapolitana   M---W---V---W---D---W---P----P---E----N----E---P-----E
T.maritimia     M---W---V---W---D---W---P----P---E----N----E---P---- E
T.Fusca         M---W---V---W---D---W---P----P---E----N----E---P-----E
S.coelicolor    M---W---V---W---D---W---P----P---E----N----E---P-----E
C.Fimi          M---W---V---W---D---W---P----P---E----N----E---P-----E
X.fastidiosa    M---W---V---W---D---W---P----P---E----N----E---P-----E
P.furiosus      M-------V---W---D---W---P----P---E----N----E---P------

Amino acid      556--653--657--671  879
residue no      507--591--595—-599  787
                507--591--585--599  785
                533--617--621--625  840
                517--601--605--609  820
                519--603--607--611  823
                576--662--666--670  891
                391--462--466--470  510

SMANB            G----G----W----D----Y
T.neapolitana    G----G----W----D----R
T.maritimia      G----G----W----D----R
T.Fusca          G----G----W----D----V
S.coelicolor     G----G----W----D----A
C.Fimi           G----G----W----D----H
X.fastidiosa     G----G----W----D----Q
P.furiosus       G-E--G----W----D----G
                   ▲
```

Figure 4. Computational comparison of a deduced amino acid sequence of human MANB with bacterial β-mannosidases. SMANB represents cloned human β-mannosidase (EU009130). Amino acid sequence alignment was done using ClustalW 1.83. The common conserved aligned amino acid sequences and the amino acid residue numbers are shown. The proposed catalytic nucleophilic (*Glu-554*) and proton donor (*Glu-457*) sites based on the human β-mannosidase are marked with a bold "**E**." Dashes indicate the nonidentical amino acid sequences. The nucleophilic catalytic site of *P. furiosus* is shifted at amino acid residue 395, shown by an arrowhead.

conserved in mammals but different among species. In mammals, a disorder of the β-mannosidase enzyme leads to β-mannosidosis, a lysosomal storage disease. The defective activity of MANB has not yet been studied in mammals other than goat, bovine, and human. In human β-mannosidosis, the splice mutation of A to G in intron 15 and the 4 base insertions of ATAA between exon 7 and 8 disturb the normal transcription process and null mutation (15,18). In humans, the amino acid sequence of MANB after methionine (the start codon) has the characteristic eukaryotic signal peptide sequence (24), which is in agreement with bovine β-mannosidase. The translation, maturation, and glycosylation processes of the human β-mannosidase enzyme are not completely known, but they can be compared with bovine β-mannosidase, which indicates the cleavage of the signal peptide at residue 17 following the (-3, -1) rule (24). The hydrophobic region in the deduced amino acid sequence of human MANB can be compared with the bovine β-mannosidase amino acid sequence, which is predicted at amino

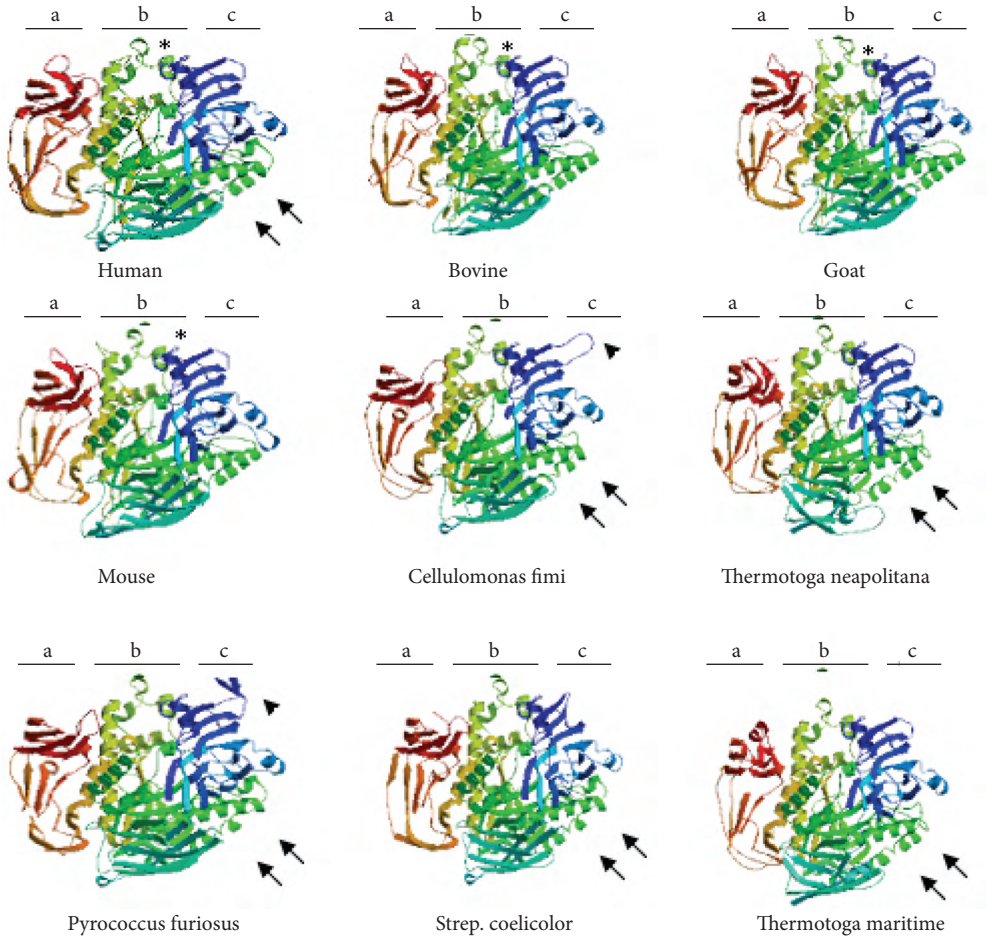

Figure 5. Prediction of 3-dimensional models of the β-mannosidase enzyme on the basis of amino acid sequence using ExPASy. Asterisk indicates an extra α-helix in bovine and goat, (►) indicates an extra loop of β-pleated sheet in *C. fimi* and *P. furiosus*, and a double arrow indicates fewer α-helices in part C of the microbial MANB.

acid residues 96-114 and 406-422 and is based on the Kyte-Doolittle hydropathy plot (25).

The biochemical functions of proteins can be best understood by predicting their 3-dimensional structures on the basis of homology modeling. The numbers of protein structures being determined today are fewer than the numbers of known protein sequences. It is estimated that during 2008, nearly 53,000 experimentally proven protein structures were deposited in the Protein Data Bank (26). This is relatively fewer than the 6 million protein sequences admitted into the Uniport knowledge database (27). Generally, the structural similarity between 2 homologous proteins is determined by the similarities of the amino acid sequences, which leads to the determination of the 3D structure of the proteins. On the basis of this homology modeling, the 3D structure of MANB of different species (prokaryotes to eukaryotes) was determined. The 3D structure provides fruitful insight for understanding the catalytic activity and mode of enzymatic reaction under different normal and pathological conditions. In MANB homology modeling, the open reading frames (ORFs) of species were used. The genomic ORFs of other species, such as *Saccharomyces cerevisiae*, *E. coli*, *M. genitalium*, *C. elegans*, and *M. jannaschii*, were used to identify the 3D structures. The homology among different sequences varied from

16% to 21% (28). The main advantages of 3D modeling are that it describes the right way of accepting or rejecting the match, that it can provide the active and binding sites of the proteins or enzymes, and that it can directly predict the functional properties of the enzymes or proteins that are not possible to elucidate only on the basis of amino acid sequence homology.

The β-mannosidosis disease is not completely understood due to lack of information about the structure and function of the β-mannosidase enzyme. In this report, the computational and 3D structural comparison of the deduced amino acid sequences of human β-mannosidases with mammalian, plant, fungal, and bacterial β-mannosidases allowed us to understand the sequence similarities, which will facilitate the identification of the structure and catalytic domain of the MANB enzyme in ongoing experiments on the differential expression of the MANB gene in cancer.

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