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A Review on the Structure-function Relationship of Class II α-Mannsoidases

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Of fundamental importance to every function an enzyme or a protein performs is its three-dimensional structure. The structure plays a crucial, though often subtle, role in catalysis. The conformational changes in enzymes are often small, and even these small changes could be significant. Glycosylation modifications of proteins and glycan hydrolysis are critical for protein function in biological processes. Aberrations in glycosylation enzymes are linked to lysosomal

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storage disorders (LSD), immune interactions, congenital disorders, and tumour progression. Alpha-Mannosidases Class 2B is a lysosomal hydrolase. Dysfunction of this has been implicated as a causative factor in mannosidosis, a lysosomal storage disorder characterized by cognitive impairment, hearing loss, and immune system and skeletal anomalies. Despite decades of research, its role in pathogenic infections, autoimmune conditions, cancers, and neurodegenerative pathologies is highly ambiguous. Although many Class II α-mannosidases have been reported from various sources but not many of them have been characterized structurally. Through the combined efforts of various types of spectroscopies and protein crystallography, structure-function relationship studies in some Class II α-mannosidases from various sources have been carried out, and the same has been reported. The structure of Class II α-mannosidases from different sources has revealed the involvement of various types of folding of the protein, the presence of metal ions such as Zn^{+} in the active site, and their role in substrate specificity and catalytic mechanism is reviewed here.

Keywords: Alpha-mannosidase; structure-function relationship; 3D-structure; spectroscopy.

1. INTRODUCTION

"In the modifications of proteins, the glycosylation is one of basic and poorly understood methods. Majority of the soluble and membrane-bound proteins synthesized in the Endoplasmic Reticulum are all glycosylated. Protein N-glycosylation takes place primarily in the endoplasmic reticulum (ER) and in Golgi apparatus, and involves a series of discrete catalytic steps. A variety of enzymes, such as glycosidases, have evolved to carry out the complex steps involved in this this pathway. The enzymes glycosidases play important functions in all organisms ranging from the degradation of sugars to the alteration of the structures of glycan moieties on the surface of proteins. In addition, they are also involved in biological processes such as the digestion, the biosynthesis of glycoproteins and the catabolism of glycoconjugates" (de Melo et al., 2006; Wolfenden et al., 1998). "In the early 1990's, classification of glycoside hydrolases, or glycosidases was first carried out based on amino acid sequences" (Henrissat, 1991). "The sequences of totally 301 known glycoside hydrolase enzymes were grouped into 35 families. The substrate specificity and reaction mechanisms of the classified enzymes corresponded to their family placement. Currently, the carbohydrate-active enzyme database (CAZy; www.cazy.org) has classified thousands of glycoside hydrolase enzymes into approximately more than one hundred families" (Coutinho & Henrissat, 1999).

"Mannosidases are glycohydrolase enzymes involved in the processing of mannose containing glycans *in vivo* and involved in final maturation and degradation of glycoprotein-linked oligosaccharides. Use of naturally occurring substrates for specificity determination, antibodies from subcellular localization, specific enzyme inhibitors and mutant cell lines, has revealed the presence of different α-mannosidase iso-enzymes in the glycoprotein metabolism" (Daniel et al., 1994). "α-Mannosidases, EC 3.2.1.24 (α-D-mannoside mannohydrolase), is involved in hydrolysis of terminal α-D-mannose residues in a

Table 1. Classification of α-mannosidase

Sl. No.	Properties	Class I α-Mannosidase	Class II α-Mannosidase
1	Substrate Specificity	α-1,2 mannoside linkages	α-1,2 α-1,3 and α-1,6 mannoside linkages
2	Artificial substrates (aryl mannosides)	No activity	Active
3	Ca^{2+}	Required for activity	Not required for activity
4	Inhibition	1-deoxymannojirimycin (dMNJ) and Kifunensine (KF)	Swainsonine and 1,4-dideoxy-1,4-imino-D-mannitol (DIM)
5	Cleavage of glycosidic linkage	By Inversion of configuration of the released mannose (22, 23)	By Retention of configuration of released mannose (24)
6	Glycohydrolase Family	47	38

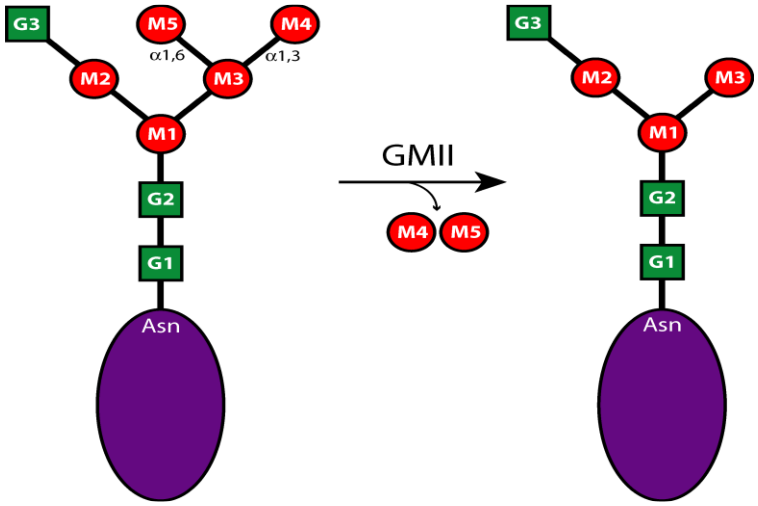

Fig. 1. Mode of catalysis of class II α-mannosidases. Diagrammatic representation of the catalytic action of class II α-mannosidases

Table 2. Comparison of Class I and II α-mannosidase

Class	Sub-class	Source	Function	Metal ion requirement	Inhibitor
I	A	Lower and higher eukaryotes	Glycoprotein breakdown by cleavage of α-1,2 linkage	Ca^{2+}	1-deoxymannojirimycin
	B	Lower and higher eukaryotes	Glycoprotein breakdown by cleavage of α-1,2 linkage	Ca^{2+}	1-deoxymannojirimycin
II	A	Higher eukaryotes	Glycoprotein synthesis	Not required with some exceptions as Jbα-man etc.	Swainsonine
	B	Higher eukaryotes	Glycoprotein breakdown by cleavage of α-1,2, α-1,3 and α-1,6 linkage		Swainsonine
	C	Lower and higher eukaryotes	Glycoprotein breakdown by cleavage of α-1,2, α-1,3 and α-1,6 linkage		Swainsonine

polysaccharide α-D-mannosides. There are two classes of processing α-mannosidases (Table 1) based on their characteristics, distinctive substrate specificity, responses to inhibitors, cation requirements, protein molecular weights, subcellular localization, enzyme mechanisms, and characteristic regions of conserved amino acid sequences" (Daniel et al., 1994; Moremen et al., 1994; Herscovics, 1999). In both the ER and Golgi of mammalian cells, the processing mannosidase enzymes are found. The differential activity of Class II α-mannosidase towards many artificial substrates has important practical utility. Because Class II mannosidase enzymes are readily assayed with aryl mannosides substrates, they are almost always employed for the initial screening of potential new inhibitors (Gonzalez & Jordan, 2000).

1.1 Subclasses of Class II α-mannosidase

The major enzymes of class II α-mannosidase that have been characterized always catalyse the degradation of Asn-linked oligosaccharides. This group of enzymes consists of three subfamilies of genes (Classes IIA, IIB and IIC) with distinct cellular functions in the system. Some properties have been summarized in Table 2. In the system, the enzymes of Class IIA subfamily is involved in N-glycan synthesis in the Golgi, while the Class IIB and Class IIC were involved in the N-glycan breakdown, removal and recycling in the cytoplasm, lysosome and vacuole.

1) The first subfamily of Class II genes (Class IIA) are responsible for removal of α-1,3- and α-1,6-linked mannose residues from N-glycans during their synthesis (Fig. 1), a process which occurs only in the higher eukaryotes, but not in lower eukaryotes, such as fungi.

2) The second subfamily (Class IIB) is found in higher eukaryotes and enzymes are involved in N-glycan degradation in the lysosome organelle.

3) The Class IIC, which is third subfamily, contains a heterogeneous set of enzymes. Sequence analysis resolves the various inter-relationships among these proteins (Eades et al., 1998). This subfamily has very low sequence similarity to the other two subfamilies. The Class IIA and Class IIB subfamilies have diverged more recently than the Class IIC subfamily as revealed by phylogenetic analysis. A single common ancestor might have duplicated after the divergence of lower eukaryotes, such as fungi, from the higher eukaryotes. Hence, the lower eukaryotes contain only the orthologue of the common ancestor. Further duplication in the higher eukaryotes led to the formation of the three subfamilies of Class II genes found in the higher eukaryotes. These gene sequences diverged and evolved more specialized functions, such as the more complex N-glycan pathways (Class IIA), and more efficient degradation pathways (Class IIB).

It is very much intriguing fact that the two classes of α-mannosidases have such similar and overlapping functions. In the N-glycans synthesis pathway of higher eukaryotes, Class I and the Class IIA α-mannosidases have complementary functions. The other Class II α-mannosidases have broad substrate specificity and they are able to cleave α-1, 2 (as well as α-1, 3 and α-1, 6) mannose linkages, which is a property that shared with the Class I genes. No sequence similarity was observed between the Class I and Class II genes and appear to have been originated independently and represent a classic case of convergent evolution (Eades & Hintz, 2000). Table 2 shows an interesting comparison between the Class I and II α-mannosidase enzymes.

Enzymes of Class II α-mannosidase occur in plants, animals, and microbes. This review summarizes the available information on the structure-functional relationship among Class II α-mannosidase enzymes.

2. MICROBIAL CLASS II α-mannosidases

For the analysis of glycopeptides and developmental regulation of lysosomal enzymes, microbial Class II α-mannosidases are generally used. Hence, here we provide a comparison of properties of this high molecular weight, oligomeric protein from a number of microbial sources (Table 3).

The earlier workers have reported the production of Class II α-mannosidase enzymes from bacteria like *Arthrobacter* (Jones & Ballou, 1969) and *Cellulomonas* (Takegawa et al., 1989). Protozoans are also known to produce Class II α-mannosidase which is involved in the processing of high mannose oligosaccharides present in the proteins. Avila *et al.*, reported the presence of 12 acid hydrolases including Class II α-mannosidase from *Trypanosoma cruzi* (Avila et al., 1979) and characterization of Class II α-mannosidase from *Trypanosoma rangeli* (Nok et al., 2000) has also been reported. *Aspergilli* genus are the major producers of the enzyme, viz., *Aspergillus niger* (Matta & Bahl, 1972),

Aspergillus saitoi (Amano & Kobata, 1986) and *Aspergillus fischeri* (Gaikwad et al., 1995; Keskar et al., 1996; Gaikwad et al., 1997; Shashidhara & Gaikwad, 2009; Shashidhara & Gaikwad, 2010). Earlier reports on Class II α-mannosidase from Dictyostellium discoideum (Shashidhara et al., 2012) and Trichoderma reesei (Schatzle et al., 1992) are also available. Class II α-mannosidases are well studied in *Saccharomyces cerevisiae* (Van Petegem et al., 2001) and *Candida albicans* (Jelinek-Kelly et al., 1985; Vazquez-Reyna et al., 1993) among the yeasts.

From Table 3, it can be observed that the biochemical parameters of the enzyme from bacteria, fungal and yeast sources are almost identical. The activity of the enzyme is optimum in the pH range of 5.0 - 7.0 and temperature 37-55°C. The enzyme is most stable in the pH range of 5.0-7.0 and up to 50°C for not more than an hour. The Class II mannosidase enzyme is oligomeric in nature and it has an iso-electric point on the acidic pH side.

2.1 *Aspergillus fischeri* Class II α-mannosidase

The Class II α-mannosidase from *Aspergillus fischeri* is one of the Class II α-mannosidases, which has been extensively studied by our research group.

"Fluorescence quenching and Time-resolved fluorescence of Class II α-mannosidase, a multi-tryptophan protein from *Aspergillus fischeri* were carried out to investigate the tryptophan environment. The tryptophan amino acids in the protein were differentially exposed to the solvent and were not fully accessible to the neutral quencher which indicates the heterogeneity in the environment" (Shashidhara & Gaikwad, 2007).

"Energetics of the catalysis of Class II α-mannosidase (E.C.3.2.1.24) from *Aspergillus fischeri* was also studied. The enzyme has showed K_{cat}/K_m for Man (α1-3) Man>, Man (α1-2) Man and> Man (α1-6) Man. The activation energy, E_a was for α1-3<, α1-2 < α1-6 linked mannobioses, respectively, reflecting the energy barrier in the hydrolysis of latter two substrates. Binding of Swainsonine to the enzyme was stronger than that of 1-deoxymannojirimycin" (Shashidhara & Gaikwad, 2009).

"The conformational transitions of an oligomeric and high molecular weight class II α-mannosidase enzyme from fungi *Aspergillus fischeri* was examined using fluorescence and CD spectroscopy under chemical, thermal, and acid denaturing conditions. The enzyme lost its activity first and then the overall folded conformation and secondary structure. The enzyme completely unfolded in 4.0 M *GdnHCl* but not at 90°C temperature. The inactivation and unfolding of the protein were irreversible in nature. The protein exhibited molten-globule-like intermediate at pH 2.0 with rearranged secondary and tertiary structures and exposed hydrophobic amino acids on the surface" (Shashidhara & Gaikwad, 2010).

"To use Class II α-mannosidase as a target against anticancer drugs, inhibition studies of a model enzyme, Class II α-mannosidase from *Aspergillus fischeri* was carried out to reveal the sensitivity of the enzyme. Three of the derivatives have shown competitive inhibition (K_i = 45, 48 and 235μM), and the binding of the inhibitors to the enzyme was found to be entropically driven. Among the metal ions, Cu^{2+} (K_i = 21nm) and Se^{2+} ions (K_i =32 μM) showed the non-competitive and Co^{2+} (K_i = 1.195 mM) showed the competitive inhibition of the enzyme activity with insignificant change in the secondary structure of the protein. The above studies exhibited the potential of this enzyme in studying the anticancer drugs. Treatment of the mannosidase enzyme with group specific reagents also showed the presence of carboxylate, Arg and Cys amino acids at the active site of the protein. The amino acids Trp and His at the active site were observed to be in the proximity" (Shashidhara et al., 2009).

"The α-mannosidase gene from *Aspergillus fischeri* was found to be 1990 base pair long and the nucleotide sequence analysis revealed the presence of three introns and four exons in the gene. BLAST analysis showed 100% similarity to the *Neosartorya fischeri* (*A. fischeri*) and 94% similarity to the *Aspergillus fumigatus* class I α-mannosidase reported in NCBI Gene Data Bank. Our previous biochemical studies on the present mannosidase enzyme have revealed that it is a class II but not class I α-mannosidase. As described earlier, class II α-mannosidases have three subfamilies of genes- Class IIA, IIB and IIC. Class IIC has very low sequence similarity to the other two subfamilies and are heterogeneous enzymes with a diversity of functions and cellular location. Hence, the α-mannosidase from

Table 3. Summary of properties of purified microbial Class II α-mannosidases

Source	Optimum pH / pH Stability	Optimum Temp. (°C) / Temp. Stability	pI	M_r (kDa)	Substrate[a]	Inhibitor[b]	References
Aspergillus Fischeri	6.0-6.5 / 6.0-6.5	50-55 / 50 °C, 30 min	4.5	412	1-2,1-3,1-6, pNPαMan, 4-MeUmbαMan	Cu^{2+}, Hg^{2+}	(Gaikwad et al., 1995; Gaikwad et al., 1997; Shashidhara & Gaikwad, 2009; Shashidhara et al., 2009; Shashidhara et al., 2012)
Arthrobacter	6.5-7.0 / 5.0-9.0	37 / 50 °C, 5 min	-	-	1-2,1-3,1-6, pNPαMan	Cu^{2+}, Hg^{2+}, Zn^{2+}	(Jones & Ballou, 1969)
Cellulomonas	6.5-8.0 / 5.0-9.5	50 / 40 °C, 10 min	3.2	450	1-2,1-3,1-6, YM	Cu^{2+}, Hg^{2+}, Zn^{2+}, EDTA	(Takegawa et al., 1989)
Saccharomyces cerevisiae	5.9-6.8 / -	- / -	-	-	1-2,1-3,1-6, pNPαMan	1-DMJ and DIM	(Jelinek-Kelly et al., 1985)
Candida Albicans	- / -	- / -	-	417	1-2,1-3,1-6, 4-MeUmbαMan	1-DMJ and Swainsonine	(Vazquez-Reyna et al., 1993; (Vazquez-Reyna et al., 1999)
Aspergillus Niger	4.8 / 5.0-8.0	45 / -	-	-	1-4, 1-6, pNPαMan,	Mannono 1-5 lactone	(Matta & Bahl, 1972)
Aspergillus Saitoi	5.0 / -	30	-	-	1-2,1-3,1-6,	Cu^{2+}	(Amano & Kobata, 1986)
Trypanosoma Cruzi	- / -	-	-	240	-	-	(Avila et al., 1979)
Trypanosoma Rangeli	5.0-6.0 / -	37	-	90	4-MeUmbαMan	Swainsonine	(Nok et al., 2000)
Dictyostelium discoideum	- / -	-	-	140	-	-	(Schatzle et al., 1992)
Trichoderma reesei	6.5 / -	-	-	160	-	-	(Van Petegem et al., 2001)

[a] The mannobioses- 1-2: Manα(1-2)Man; 1-3: Manα(1-3)Man; 1-6: Manα(1-6)Man, pNPαMan: p-nitrophenylα-D-mannopyranoside, 4-MeUmbαMan: 4-methylumbelliferrylα-D-mannopyranoside, YM: Yeast mannan.

[b] 1-DMJ: 1-deoxymannojirimycin, DIM: dideoxy-1,5-imino-D-mannitol.

Aspergillus fischeri belongs to the Class IIC subfamily" (Shashidhara et al., 2012).

3. PLANT

In Table 4, reports on α-mannosidases from plant sources are mentioned and jack bean α-mannosidase is the most studied among for all of its characteristics.

The enzymes from plant sources exhibits distinguishing feature of requiring Zn^{2+} or Co^{2+} for most of the enzymes. As compared to that from microbial sources the optimum pH of the enzyme lies slightly on the acidic side. Details on biochemical and biophysical characterization of Class II α-mannosidases have been carried out only on the enzyme from *Canavalia ensiformis*.

3.1 *Canavalia ensiformis* Class II α-mannosidase

This enzyme was extensively used to explore the linkage of mannose in several glycoproteins (Li, 1967). The enzyme hydrolyzes mannobiose, mannotriose, and mannotetraose derived from yeast mannan. The jack-bean α-mannosidase can be considered as a metalloenzyme since it is existing naturally as a zinc-protein complex (Snaith, 1975). "Jack bean α-mannosidase had wide acceptor substrate specificity and could transfer mannosyl residues to various acceptors such as D-fructose, L-arabinose, maltose, lactose, and sucrose" (Hara et al., 1994). "The enzyme is a retaining Glycohydrolase type. It would provide a useful model system in mechanistic studies and inhibitor design as it was shown to be mechanistically similar to the lysosomal enzyme" (Howard et al., 1997). Two new mechanism-based inhibitors, 5-fluoro-α-D-mannosyl fluoride and 5-fluoro-β-L-gulosyl fluoride, which function by the steady state trapping of such an intermediate, were synthesized and tested. Both show high affinity but only the latter has been used to label the active site nucleophile. "Comparative liquid chromatographic/mass spectrometric analysis of peptic digests of labelled and unlabeled enzyme samples confirmed the unique presence of this peptide of m/z 1180.5 in the labelled sample. They have shown presence of conserved amino acid i.e., Aspartic acid residue at active site, contained within the peptide sequence Gly-Trp-Gln-Ile-Asp-Pro-Phe-Gly-His-Ser, which showed excellent sequence similarity with regions in mammalian lysosomal and Golgi α-mannosidase sequences, family 38 class II α-mannosidases in which the Asp in the above sequence is totally conserved" (Howard et al., 1998). "Mechanism of inhibition of this enzyme by inhibitor swainsonine has also been reported" (Kang & Elbein, 1983).

"The conformational transitions in a multimeric and high molecular weight class II a-mannosidase from Jack Bean (*Jbα-man*) were examined utilizing enzyme properties like intrinsic fluorescence, solute quenching, hydrophobic dye binding, size exclusion chromatography and circular dichroism (CD) spectroscopy for the protein in presence of Guanidine hydrochloride (GdnHCl). The decomposition analysis of the protein spectra obtained during unfolding showed progressive appearance of class S, I, II and III trp. The parameter A and spectral center of mass showed multi-state unfolding of the protein and phase diagram analysis revealed formation of an intermediate of *Jbα-man* in the vicinity of 1 M GdnHCl solution. The intermediate protein exhibited compact secondary and distorted tertiary structure with exposed hydrophobic amino acids on the surface, indicating the molten-globule nature. The dissociation, partial unfolding and aggregation of *Jbα-man* occurred simultaneously during chemical denaturation. Unlike the native *Jbα-man,* the molten-globule possessed slightly higher hydrodynamic radius, perturbance in the structure up to 60°C and stability of the structure up to 80°C. The modes of chemical and thermal denaturation of the native protein were different. The solute quenching parameters confirmed the altered confirmation of the intermediate. Taken together, these results constitute one of the early reports of formation of GdnHCl induced molten globule in a class II α-mannosidase" (Kumar & Gaikwad, 2010).

The jack bean α-mannosidase (*Jbα-man*) enzyme was maximally stable at pH 5.0; however, when incubated in the pH range of 11.0–12.0, it has showed 1.3 times higher activity and also stability for longer time. The free amino group at or near the active site of this enzyme was probably involved in the stability and activation mechanism. The active site was observed to be constituted by the association of two un-identical subunits connected mainly by disulfide linkages. The metalloenzyme has Zn^{2+} ions tightly bound to it and chelation reduces the thermal stability of the protein. Energetics of catalysis and thermodynamics of inhibition of this enzyme has showed that the entropy driven strong binding occurs with the inhibitor, Swainsonine (Kumar & Gaikwad, 2011).

Table 4. Summary of properties of purified plant Class II α-mannosidases

Source	Mol.Wt. (kDa)	Subunit Mol. Wt. (kDa)	Optimum pH / Optimum Temp. (°C)	K_m (mM)	Metal ion requirement	Inhibitor	Substrate[a]	Reference
Jack bean	230	66 and 49	5.0 / 45	-	Zn^{2+}	Cu^{2+}, Co^{2+}, Cd^{2+} and Swainsonine	1-2,1-3,1-6, pNPαMan, 4-MeUmbαMan	(Li, 1967; Snaith, 1975; Hara et al., 1994; Howard et al., 1998; Kumar & Gaikwad, 2011)
Mung bean	-	125	6.0 / -	-	-	Swainsonine	1-3,1-6, pNPαMan	(Suvarnalatha & Prabha, 1999)
Tomato	-	38	4.5 / 65	1.08	-	-	pNPαMan	(Kuokkanen et al., 2007)
Capsicum	43	23	5.7 / 50	0.7	-	Cu^{2+} and Fe^{2+}	pNPαMan	(Heikinheimo et al., 2003)
Rice	-	-	4.3-4.5 / -	1.04	Zn^{2+}	EDTA, Swainsonine	1-2,1-3,1-6, pNPαMan	(Mathur et al., 1984)
Gingko biloba	340	120	- / -	-	Co^{2+}	Ca^{2+}	high-mannose type free *N*-glycans	(Merkle et al., 1997; Numao et al., 2003)
Phaseolus vulgaris	194	110	4.6 / -	1.6	Zn^{2+}	-	pNPαMan	(Van Den Elsen et al., 2001; Oku et al., 1991; Yamashiro et al., 1997)
Almond	-	-	3.8 / 60	-	-	-	pNPαMan	(Sun & Wolfe, 2001)
Babaco	260-280	-	4.5 / 50-60	1.25	-	-	pNPαMan	(Suits et al., 2010)
Erythrina indica	-	-	- / -	-	Zn^{2+}	-	pNPαMan	(Kestwal & Bhide, 2005; Kestwal et al., 2007)
Lentil	489	130	5.0 / 55	4	No	Cu^{2+}, Co^{2+}, Hg^{2+} and Swainsonine	pNPαMan, 4-MeUmbαMan	Unpublished data

[a] The mannobioses- 1-2: Manα(1-2)Man; 1-3: Manα(1-3)Man; 1-6: Manα(1-6)Man, pNPαMan: p-nitrophenylα-D-mannopyranoside, 4-MeUmbαMan: 4-methylumbelliferrylα-D-mannopyranoside.

Table 5. Summary of properties of purified animal Class II α-mannosidases

Source	Mol.Wt. (kDa)	Subunit Mol. Wt. (kDa)	Optimum pH	K_m (mM)	Metal ion requirement	Inhibitor	Substrate[a]	Reference
Human	200	-	4.5	2.7	Fe^{2+}, Co^{2+} and Mn^{2+}	Cu^{2+} and Swainsonine	High-mannose type free *N*-glycans and pNPαMan	(Ockerman, 1967)
Bovine	-	-	-	-	-	-	-	(Burditt et al., 1978)
Monkey	-	-	-	-	-	-	-	(Bischoff et al., 1986)
Murine	160-180	-	4.5	12.6	-	Swainsonine	-	(Misaki et al., 2003)
Drosophila	-	-	-	-	-	-	-	(Zymenex et al., 2005; Winkler & Holan, 1989)
Japanese quail	330	-	7.0	3	Co^{2+}	Cu^{2+}, Hg^{2+}, Swainsonine and 1-DMJ	High-mannose type free *N*-glycans and pNPαMan	(Agrawal & Bahl, 1968)
Hen	480	110	7.0	0.44	Co^{2+}	Zn^{2+}, Cu^{2+} and Hg^{2}	High-mannose type free *N*-glycans and pNPαMan	(Jagadeesh et al., 2004)

3.2 *Erythrina indica* Class II α-mannosidase

α-mannosidase from *Erythrina indica* seeds (Kestwal & Bhide, 2005) is "a Zn^{2+}-dependent glycoprotein with 8.6% carbohydrate content. The energy of activation of the enzyme was found to be 23 kJ mol^{-1}. N-terminal sequence known to contain amino acid sequences such as Asp, Thr, Gln, Glu, and Asn. Treatment of the enzyme with N-bromo succinimide (NBS) led to total loss of enzyme activity due to the modification of a single tryptophan residue. The enzyme exhibited immunological identity with α-mannosidase from *Canavalia ensiformis* but not with the same enzyme from *Glycine max* and *Cicer arietinum*. Incubation of *E. indica* seed lectin with α-mannosidase resulted in 35% increase in its enzymatic activity. Lectin induced activation of α-mannosidase could be completely abolished in presence of lactose, which is a sugar specific for lectin" (Kestwal et al., 2007).

3.3 *Lycopersicon esculentum* Class II α-mannosidase

"α-Mannosidase from tomato (*Lycopersicon esculentum*) was found to contain two isoforms of class II α-mannosidase, isoform I and II, which were purified by ion exchange and gel filtration chromatography, showing 6% and 24% of the total activity, respectively. Both the enzymes had pH optima of 4.5 and were thermally stable at 65 °C for up to 15 min. The Km values for pNPαman were 1.11 and 1.05 mM, respectively. Purified isoform II had a SDS Mr of ca. 38,000" (Suvarnalatha & Prabha, 1999).

4. ANIMAL

Animal sources of α-mannosidase include humans (Kuokkanen et al., 2007) and bovine (Heikinheimo et al., 2003) which form very important source for the study of the enzyme widely. Apart from these, α-mannosidase has also been reported from monkey brain (Mathur et al., 1984), murine (Merkle et al., 1997), *Drosophila melanogaster* (Numao et al., 2003; Van Den Elsen et al., 2001), Japanese quail oviduct (Oku et al., 1991), hen oviduct (Yamashiro et al., 1997) etc.

In humans and cattle, lack of lysosomal α-mannosidase activity causes the autosomal recessive disease called α-mannosidosis. Lysosomal α-mannosidase is a major exoglycosidase in the glycoprotein degradation pathway. Recently, great progress has been made in studying the enzyme and its deficiency. This includes cloning of the gene encoding the enzyme, characterization of mutations related to the disease, establishment of valuable animal models, and encouraging results from bone marrow transplantation experiments (Sun & Wolfe, 2001).

These enzymes do act in the neutral pH range. The enzyme from human, monkey brain and murine source have been cloned and significant homology in the gene sequence has been observed. Fe^{2+} and Co^{2+} are important metal cofactors for the enzyme from human, Japanese quail oviduct and Hen oviduct.

The important feature of the structural intermediate formed during reaction was elucidated from three-dimensional structure of α-mannosidase from *Drosophila melanogaster*. Distorted skew boat conformation was seen in the resolution at 1.2 Å.

4.1 Human

"A neutral alpha-mannosidase has been proposed to be involved in hydrolysis of cytosolic free oligosaccharides originating either from ER-misfolded glycoproteins or the N-glycosylation process. Although this enzyme was isolated from the cytosol, it has also been linked to the ER by subcellular fractionations. The purified recombinant enzyme was found to be a tetramer and has a neutral pH optimum for activity. It hydrolyzed $Man_9GlcNAc$ to $Man_5GlcNAc$ in the presence of Fe^{2+}, Co^{2+} and Mn^{2+} and uniquely to neutral alpha-mannosidases from other organisms; the human enzyme was more activated by Fe^{2+} than Co^{2+}. These findings from enzyme-substrate characterizations and subcellular localization studies support the suggested role for neutral alpha-mannosidase in hydrolysis of soluble cytosolic oligomannosides" (Kuokkanen et al., 2007).

4.2. Bovine

"The three-dimensional structure of bovine lysosomal α -mannosidase (*bLAM*) was reported. It offered indications for the signal areas for mannose phosphorylation and suggested a previously undetected mechanism of low-pH activation and provides a template for further biochemical studies of the family 38 glycoside hydrolases as well as lysosomal transport. Furthermore, it provided a basis for understanding the human form of α -mannosidase at the atomic level" (Heikinheimo et al., 2003).

4.3. Monkey

"Lysosomal α-D-mannosidase of monkey brain was found to exist in two forms. One form of α-mannosidase was found to bind to RCA1-Sepharose and could be specifically eluted with lactose. The other form did not bind to the RCAl-Sepharose. Both forms of mannosidase were found to bind to a similar extent to the immobilized brain lysosomal receptor protein. Both the forms were purified to apparent homogeneity. Neutral sugar analysis by GLC showed the presence of glucose, mannose and galactose in the RCAl-Sepharose bindable mannosidase and glucose and mannose in the non-bindable mannosidase" (Mathur et al., 1984).

5. CRYSTAL STRUCTURE OF CLASS II α-mannosidases

Not many three-dimensional structures of class II α-mannosidases are known. Those known are of Bovine lysosomal α-mannosidase (Heikinheimo et al., 2003), *Drosophila* Golgi α-mannosidase II (Numao et al., 2003) and *Streptococcus Pyogenes* α-mannosidase II (Suits et al., 2010).

The first structure of a GH38 enzyme was that of Bovine lysosomal α-mannosidase (Fig. 2A). It suggested a previously undetected mechanism of low-pH activation and also provided a template for further biochemical studies of the family 38 glycoside hydrolases (Heikinheimo et al., 2003).

"The most extensively studied of these enzymes is the *Drosophila* GH38 α-mannosidase II, which has been shown to be a retaining α-mannosidase that targets both α-1,3 and α-1,6 mannosyl linkages, an activity that enables the enzyme to process $GlcNAcMan_5GlcNAc_2$ hybrid *N*-glycans to $GlcNAcMan_3GlcNAc_2$. The crystal structure of Drosophila Golgi α-mannosidase II (Fig. 2B) revealed a novel protein fold with an active site zinc intricately involved both in the substrate specificity of the enzyme and directly in the catalytic mechanism" (Numao et al., 2003; Van Den Elsen et al., 2001).

"Streptococcus pyogenes GH38 enzyme (*SpGH38*) is an α-mannosidase with specificity for α-1, 3 mannosidic linkages. The 3D X-ray structure of *SpGH38* (Fig. 2C), obtained in native form at 1.9 Å resolution, revealed a canonical GH38 five-domain structure in which the catalytic "-1" subsite showed high similarity with the *Drosophila* enzyme, including the catalytic Zn^{2+} ion. In contrast, the "leaving group" subsites of *SpGH38* displayed considerable differences to the higher eukaryotic GH38s; features that contribute to their apparent specificity" (Suits et al., 2010).

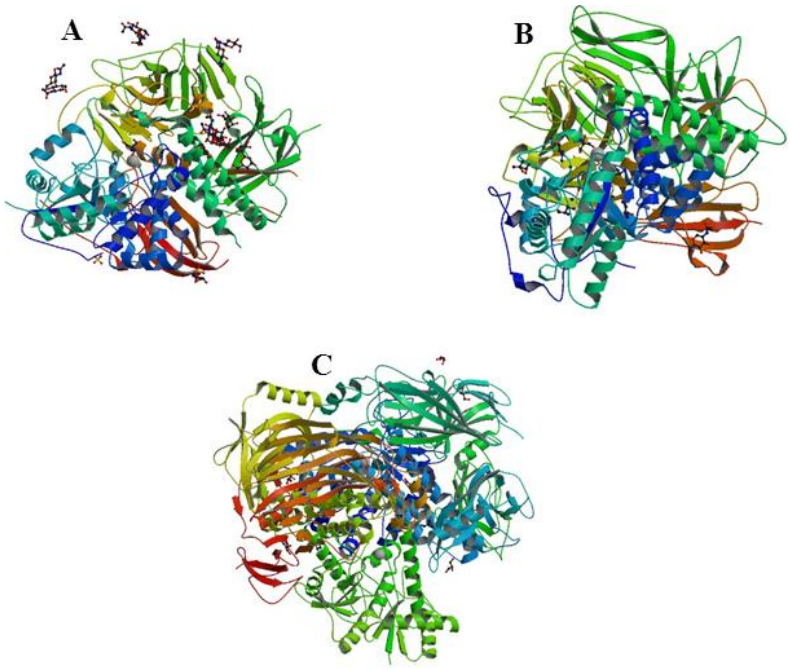

Fig. 2. Reported crystal structure of class II α-mannosidases. 3D crystal structure of class II α-mannosidases from (A) Bovine, (B) Drosophila melanogaster Golgi complex and (C) Streptococcus

6. APPLICATIONS OF CLASS II α-mannosidase

"Study of the biological function of glycoprotein glycans is rapidly emerging as a field of cell biology. Class II α-Mannosidase (α-D-mannoside mannohydrolase, E.C. 3.2.1.24) is known to play an important role in the processing of mannose containing glycans *in vivo,* because a deficiency of the enzyme results in the lethal disease called mannosidosis, a hereditary disease reported in humans" (Ockerman, 1967) and cattle (Burditt et al., 1978).

- α-Mannosidases have been employed in the analysis of mannose containing glycans (e.g. high mannose-type sugar chains of glycoproteins) and glycolipids containing α linked mannoside residues (Bischoff et al., 1986).
- To elucidate the biological role and structures of the carbohydrate moieties of the mannoproteins, a highly specific α-mannosidase active on the polymannose component is required. Almond α-mannosidase as well as jack bean α-mannosidase have been used for analysis of sugar chain structures (Misaki et al., 2003).
- The new invention provides means and strategies for treating the lysosomal storage disorder α-mannosidosis by enzyme replacement therapy. In particular, the reduction of stored neutral mannose-rich oligosaccharides takes place in cells within the central nervous system. Accordingly, the lysosomal α-mannosidase used for the preparation of a medicament for reducing the intracellular levels of neutral mannose-rich oligosaccharides in cells within one or more regions of the central nervous system (Zymenex et al., 2005).
- Golgi α-mannosidase II is a target for inhibition of growth and metastasis of cancer cells. Golgi α-mannosidase inhibitor Swainsonine acts as anti-cancer agent (Winkler & Holan, 1989).
- And also, α-mannosidase inhibitors and their analogs were utilized to design potential anti-HIV agents (Numao et al., 2003).
- In plants, increased levels of mannosidase have been reported during the seed germination and fruit ripening (Agrawal & Bahl, 1968). Jagadeesh *et al.*, reported "the increase in the activity of α-mannosidase in tomato during fruit ripening which is significant in the context of involvement of this enzyme in deglycosylation of glycoproteins, resulting in release of free *N*-glycans. Free *N*-glycans (and hence *N*-glycoproteins) have a role in fruit ripening in tomato, where tunicamycin application to mature green fruit prevented both ripening and softening of the fruit" (Jagadeesh et al., 2004).

7. CONCLUSION

Of the α-mannosidase enzyme studied and characterized so far, Class I α-mannosidases score way over the Class II α-mannosidases. The Class II α-mannosidases studied include those from microbes, plants as well as animals but none of them have been extensively characterized. All in all, there is a dearth of structure-function relationship studies of this class of enzymes. To further support the application of Class II α-mannosidases in the development of anticancer therapy and certain other applications along with furthering contributions to the fields of enzymology, protein biochemistry and protein folding, there is a need for more structural characterization as well as crystal structure determination for the Class II α-mannosidases. Our group at National Chemical Laboratory, Pune, India has made a significant contribution by studying Class II α-mannosidases for the past 10 years and taken a significant step towards this by reporting conformational transition and other biochemical and biophysical studies of Class II α-mannosidase from *Aspergillus fischeri*, a microbial source as well as *Canavalia ensiformis* (Jack bean), a plant source.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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