Structure of Mouse Golgi α-Mannosidase IA Reveals the Molecular Basis for Substrate Specificity among Class 1 (Family 47 Glycosylhydrolase) α,1,2-Mannosidases*

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Three subfamilies of mammalian Class 1 processing α,1,2-mannosidases (family 47 glycosidases) play critical roles in the maturation of Asn-linked glycoproteins in the endoplasmic reticulum (ER) and Golgi complex as well as influencing the timing and recognition for disposal of terminally unfolded proteins by ER-associated degradation. In an effort to define the structural basis for substrate recognition among Class 1 mannosidases, we have crystallized murine Golgi mannosidase IA (space group P21_21_2), and the structure was solved to 1.5-Å resolution by molecular replacement. The enzyme assumes an (αα), barrel structure with a Ca²⁺ ion coordinated at the base of the barrel similar to other Class 1 mannosidases. Critical residues within the barrel structure that coordinate the Ca²⁺ ion or presumably bind and catalyze the hydrolysis of the glycone are also highly conserved. A Man₉GlcNAc₂ oligosaccharide attached to Asn⁵¹⁵ in the murine enzyme was found to extend into the active site of an adjoining protein unit in the crystal lattice in a presumed enzyme-product complex. In contrast to an analogous complex previously isolated for Saccharomyces cerevisiae ER mannosidase I, the oligosaccharide in the active site of the murine Golgi enzyme assumes a different conformation to present an alternate oligosaccharide branch into the active site pocket. A comparison of the observed protein-carbohydrate interactions for the murine Golgi enzyme with the binding cleft topologies of the other family 47 glycosidases provides a framework for understanding the structural basis for substrate recognition among this class of enzymes.

The processing of Asn-linked oligosaccharides in mammalian organisms is initiated in the endoplasmic reticulum by the action of α-glucosidases and α-mannosidases that cleave the Glc₃Man₉GlcNAc₂ structure primarily to a specific Man₉GlcNAc₂ isomer (Man₉B)³ (Fig. 1). Following transport to the Golgi complex, further trimming of α,1,2-mannose residues results in the production of a Man₉GlcNAc₂ structure (1). These trimming reactions are followed by additional modifications, catalyzed by glycosyltransferases and hydrolases, to yield the array of complex structures on cellular and secreted glycoproteins (2).

The Class 1 mannosidases (family 47 glycosidases) play several roles in the oligosaccharide-trimming reactions in the ER and Golgi (for reviews, see Refs. 1 and 3–7). Three subfamilies of mammalian Class 1 mannosidases have been identified; the ER mannosidase I subfamily cleaves a single residue from Man₉GlcNAc₂ to generate the Man₈B structure (Fig. 1, A and B) (1), the Golgi mannosidase I subfamily cleaves Man₉₋₈GlcNAc₂ structures to Man₉₋₇GlcNAc₂ (Fig. 1, A and C), and the EDEM subfamily of mannosidase-related proteins does not appear to have an intrinsic hydrolase activity but appears to be required for disposal of terminally misfolded glycoproteins by ER-associated degradation (ERAD) (8–13). ER mannosidase I has also been shown to play a critical role in ERAD by acting as a timing step to create a key Man₉₋₇GlcNAc₂ isomer required for initiating the disposal process (14–16). Recent data indicate that further processing to structures smaller than Man₉₋₇GlcNAc₂ may also be required for ERAD, indicating that the Golgi family of mannosidases may play a role in ERAD (16–19).

In comparing the substrate specificities of ER and Golgi mannosidase I, these enzymes were found to have differences in both degree of mannose trimming as well as branch specificity for substrate recognition. ER mannosidase I preferentially cleaves the α,1,2-mannose residue on the central branch of the Man₉₋₈GlcNAc₂ structure (residue M10; Fig. 1B) to produce the Man₈B isomer (3, 20–22). In contrast, the Golgi mannosidase I family members (designated IA, IB, and IC) preferentially cleave residue M11 or M9, followed by residue M8 (Fig. 1C (23, 24)). The order of initial mannose removal (M11 versus M9) varies among the Golgi mannosidase I family members, but all of the mammalian family members tested thus far have a relatively poor efficiency for cleavage of the α,1,2-mannose designated M10 (the target of ER mannosidase I action). Thus, the ER and Golgi mannosidase I families of enzymes have complementary and largely nonoverlapping substrate specificities, despite their similarities in protein sequence and presumed protein fold (1).

The structural basis for glycone recognition by Class 1 mannosidase IA of mammalian Golgi (see Fig. 1) requires the central branch mannose residue to have been removed from the standard Man₉GlcNAc₂ structure (see Fig. 1); dMNJ, 1-deoxymannojirimycin; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated degradation; EDEM, ER degradation-enhancing α-mannosidase-like protein; NAG, N-acetylglucosamine; MES, 4-morpholineethanesulfonic acid; r.m.s., root mean square.

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The atomic coordinates and structure factors (code 1NXC) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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‡ The abbreviations used are: Man₈B, an isomer of Man₉₋₈GlcNAc₂; dMNJ, 1-deoxymannojirimycin; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated degradation; EDEM, ER degradation-enhancing α-mannosidase-like protein; NAG, N-acetylglucosamine; MES, 4-morpholineethanesulfonic acid; r.m.s., root mean square.
nosidases was revealed through the co-crystallization of human ER mannosidase I with the mannose mimics, 1-deoxymanojirimycin (dMNJ) and kifunensine (Fig. 1D) (25). The inhibitors were found to occupy the mannose site (based on established glycosidase subsite nomenclature (26)) through a direct coordination of the O-2 and O-3 hydroxyls to an enzyme-bound calcium ion and a collection of hydrogen bonds and hydrophobic interactions that stabilized the binding of the inhibitors in the equivalent of an unusual 1C4 sugar conformation.

The structural basis for the branch specificity in substrate recognition by ER mannosidase I was previously described for the enzyme from *Saccharomyces cerevisiae* (27). The x-ray structure of this enzyme revealed an oligosaccharide attached to one protein unit of the crystal lattice extending into the active site of an adjoining protein molecule in a proposed enzyme-product complex. Sugar residues of the oligosaccharide extended from the proposed enzyme subsite, with the mannose subsite unoccupied. A key Arg residue (Arg273), conserved among the ER mannosidase I subfamily of enzymes, was found to interact with several residues in the substrate and contribute to branch specificity. These interactions with Arg273, along with other hydrogen bonding interactions with the glycan across the oligosaccharide binding cleft, resulted in the insertion of residue M7 into the +1 binding site. This enzyme-product complex presumably mimics the insertion of residues M7 and M10 of the nascent Man9GlcNAc2 structure into the respective +1 and −1 binding sites during a catalytic cycle that would result in glycoside bond cleavage and the production of the Man8GlcNAc2 isomer (28). With prolonged incubation, each enzyme is able to cleave all of the α,2-mannose residues to generate the identical Man9GlcNAc2 structure that is produced in vivo (23, 59). The mannose residue numbering and color scheme is the same in Figs. 4–6. D, the schematic structures of α,β-mannose in comparison to the two Class 1 mannosidase inhibitors 1-deoxymanojirimycin and kifunensine.

In an effort to define the structural basis for substrate recognition among the Golgi mannosidase IA subclass of enzymes, we chose to study murine Golgi mannosidase IA (23, 31, 32). The structure of this enzyme revealed a protein fold and catalytic site that was similar to other Class 1 mannosidases. Surprisingly, the single O-glycan on the murine glycoprotein was found to extend into the +1 mannose position of the enzyme binding site in an adjoining protein unit in the crystal lattice in a presumed enzyme-product complex analogous to the...
structure of *S. cerevisiae* ER mannosidase I. In contrast to the yeast enzyme structure, an alternative oligosaccharide conformation was bound to the active site pocket, and a distinct monosaccharide was found in the presumed +1 binding site. A comparison of the geometry and topology of the oligosaccharide binding cleft between the various Class-I mannosidases indicates that ER and Golgi mannosidases have a high potential for self-association and binding resulting from substrate interactions in a constrained binding cleft. Comparisons of the glycan structures bound in the active sites of the yeast and murine enzymes provide insights into the structural basis for substrate recognition among these Class I mannosidases.

**MATERIALS AND METHODS**

**Golgi ManIA Purification and Crystallization**—The cDNA encoding the soluble catalytic domain of mouse Golgi mannosidase IA (31) was subcloned into the *Pichia pastoris* expression vector, pHIL-51, as a fusion protein with the yeast α-factor signal sequence (23). The final construct was transformed into the GS115 *Pichia* host strain by selection for reversion in His auxotrophy as previously described (23). The strain was grown in a 100-ml shake flask culture in BMGY medium overnight and used to inoculate a 7-liter culture in minimal medium (33) in a New Brunswick BioFlow 3000 fermentor. After growth to a cell density of 60,000 g/ml using glycerol as a carbon source, the culture was starved for ~10 h and grown on 0.5 mM MeOH as an inducing agent and carbon source for 3 days (maintained using a MeOH probe and monitor (Raven Biotech) to control feed rate). The medium was harvested and clarified by centrifugation at 6000 rpm for 20 min. The enzyme was purified in batches of 2 liters by dialysis against 8 liters of 10 mM sodium succinate (pH 6.5) using 24,000 molecular weight cut-off dialysis tubing with two changes of buffer. Prior to column chromatography, the dialysate was adjusted to pH 5.5 with HCI. The dialyzed medium was loaded at 10 ml/min onto an SP-Sepharose column (1.5 × 20 cm) pre-equilibrated with Buffer A (10 mM sodium succinate, pH 5.5), washed with >100 ml of Buffer A, and eluted with a gradient of 0–0.5 M NaCl in Buffer A. Fractions containing mannosidase activity were pooled, adjusted to 1.0 M (NH₄)₂SO₄ and pH 6.5 with 0.1 M Na-HEPES buffer. The pooled enzyme sample was then loaded onto a phenyl-Sepharose column (1.5 × 20 cm); washed with 1.0 M (NH₄)₂SO₄, 10 mM Na-HEPES (pH 6.5); and eluted using a linear gradient of 1.0 to 0.5 M (NH₄)₂SO₄ in 10 mM Na-HEPES (pH 6.5). Fractions containing enzyme activity were pooled and concentrated using an Amicon YM10 membrane following the addition of NDSB-201 (Calbiochem) to 0.75 mM. Aliquots of 5 ml of concentrated enzyme sample were applied to Superdex-75 column (1 × 100 cm) pre-equilibrated with 20 mM Na-HEPES (pH 6.5), 150 mM NaCl, 5 mM CaCl₂, 0.75 M NDSB-201. The final purified enzyme was concentrated to 30 mg/ml in the same buffer and stored at -4°C.

The enzyme was screened for crystallization conditions as previously described (32) using a microbatch technique. The best crystals were obtained using precipitant conditions of 15–20% polyethylene glycol 4000 and pH 4.5–6.5. Co-crystallization of the enzyme in the presence of dMNJ was attempted using the hanging drop vapor diffusion method. For crystallization, the above enzyme solution (19 μl) was incubated with 1 μl of a 200 mM stock solution of dMNJ (Oxford GlycoSystems) at 37°C for 2 h. The hanging drop was then prepared by mixing an equal volume of protein/dMNJ solution (1 μl) with a crystallization solution containing 100 mM MES, 100 mM Tris-HCl, 25–35% polyethylene glycol 4000, pH 6.0. Single crystals were formed in 2 days at 18°C and used for structure determination.

**Data Collection**—Data were collected with a Smart 6000 CCD detector (Bruker AXS) on an FRD rotating copper anode source equipped with Hillex® optics (Rigaku/MSC). Data collection was carried out in three passes with 600 images collected in each pass (Δω = 0.3°) at a crystal to detector distance of 9 cm. For pass 1, 2θ = 25° and φ = 0°; for pass 2, 2θ = 35° and φ = 90°; for pass 3, 2θ = 0° and φ = 0°. Integration and scaling were performed with the Proteum software suite (Bruker AXS). The structure was solved by molecular replacement using the CNS software suite (34) using the coordinates of human ER mannosidase I as a probe (Protein Data Bank code 1FMI) (25). Simulated annealing refinement (CNS) was followed by automated model rebuilding with ARWARP (35). Manual rebuilding (Xfit) (36) was repeatedly iterated with refinement of coordinates and isotropic temperature factors (Refmac5 (37) and CCP4 (38)). Anisotropic refinement of temperature factors was introduced once the crystallographic residual was reduced to 17.1% (19.0% for free reflections) and resulted in only a slight drop of the residuals and improved conformance with geometrical parameters (39). Protein structural alignments and r.m.s. deviation measurements were generated using Swiss-PdbViewer (version 3.7) (40). All protein and glycan structure figures were prepared using MacPymol (version 0.95; Delano, W. L. Pymol Molecular Graphics System (2002) available on the World Wide Web at www.pymol.org) to generate rasterized images.

**RESULTS**

**Expression, Purification, and Crystallization of Golgi Mannosidase IA**—The soluble catalytic domain (amino acid residues 66–655 (31)) of murine Golgi mannosidase IA was expressed as a secreted form in the recombinant fungal host, *P. pastoris* (23). Expression was accomplished by fermentation in minimal medium with initial growth in glycerol as a carbon source followed by growth and induction using methanol as a carbon source once high cell density was achieved (32). Optimized fermentation strategies using this recombinant host have routinely yielded as much as 100 mg/liter crude recombinant enzyme in the culture medium. Chromatographic purification using a combination of ion exchange, phenyl-Sepharose, and gel filtration columns resulted in the isolation of the homogenous enzyme. An important element in the purification strategy was the inclusion of NDSB-201 in all of the buffers following the phenyl-Sepharose step through to protein crystallization. This compound has been found to aid in blocking protein aggregation during *in vitro* protein folding and crystallization studies (42–45, 47, 48). Significant losses in the recovery of recombinant Golgi mannosidase IA by aggregation and precipitation were avoided by inclusion of NDSB-201 in all of the buffers.

Previous purification and crystallization of recombinant mouse Golgi mannosidase IA under different conditions (32) resulted in crystals that diffracted to 2.8 Å (space group P2₁2₁2₁, unit cell dimensions a = 54.9 Å, b = 135.1 Å, c = 69.9 Å). The structure of this prior crystal form was not solved. In the present study, the crystals were obtained with a P2₁2₁2₁ space group (unit cell dimensions a = 55.3 Å, b = 72.2 Å, c = 129.6 Å) and diffracted to 1.51 Å (Table I). A single monomer was found in the asymmetric unit. The structure was determined by molecular replacement using the human ER mannosidase I structure (25) as a probe, followed by simulated annealing, automated and manual model building, and anisotropic refinement of temperature factors to an R_{free} of 17.1 and an R_{cryst} of 9.0. The calcium ion and oligosaccharide structure were modeled into the remaining densities following the construction of the protein model. The structure model traces a continuous amino acid sequence from residues 178–644 indicating that 112 NH₂-terminal and 11 COOH-terminal amino acids were cleaved from the recombinant protein during expression and purification. NH₂-terminal protein sequence data, SDS-PAGE
The calcium ion bound at the base of the barrel is shown as a blue sphere. The single Asn-linked oligosaccharide (Man\textsubscript{6}GlcNAc\textsubscript{2}) is site-attached to Asn\textsubscript{122} and is shown in a yellow stick representation. In the crystal lattice, the Asn-linked oligosaccharide structure bridges between protein subunits and docks in a funnel-shaped opening at the end of the barrel of an adjoining enzyme molecule in a presumed enzyme-product complex. The coloring of the helices and numbering of the helical segments are the same as shown in Fig 3A. Loops between the inner and outer helical segments in the (αα\textsubscript{2}) barrel are simple hairpins on the short chain side of the barrel (SC side), whereas extended loops and β-strand segments are found as connections between helices in the long chain side of the barrel (LC side). The NH\textsubscript{2} and COOH terminals are indicated by N and C, respectively, in each protein unit.

Overall Structure of the Molecule—The overall structure of the molecule consists of an (αα\textsubscript{2}) barrel (Fig. 2), similar to other family 47 glycosylhydrodrolases (25, 27, 29, 30), with consecutively alternating antiparallel pairs of helices forming a concentric set of inner and outer helical layers in the barrel structure. One end of the barrel has hairpin loop crossovers between the alternating inner and outer helices (short connection side (SC side), Fig. 2), and this side of the barrel also contains a COOH-terminal β-hairpin that extends into the mouth of the cylinder to form a plug at the bottom of the cavity. Residue Thr\textsubscript{635}, at the apex of the β-hairpin in the interior of the barrel cavity, is directly involved in coordination of a calcium ion in the core of the barrel (through the carbonyl oxygen and Oγ). Other conserved acidic residues in the interior of the barrel cavity are involved in forming a hydrogen-bonding network through six water molecules to result in a calcium ion containing an 8-coordinate pentagonal bipyramid geometry common to other family 47 glycosidases (25, 27, 29, 30). A single disulfide bond is found between Cys\textsubscript{478} and Cys\textsubscript{510} to bridge helices α10 and α11. An equivalent conserved and essential disulfide is found in several other family 47 glycosidases (25, 27, 30), whereas these residues are replaced with other amino acids in the α1,2-mannosidases from T. reesei (29), indicating that the disulfide bridge at this position is not essential in all family 47 glycosidases.

The overall conservation of the protein fold among the family 47 glycosidases is indicated by the high degree of primary sequence conservation, particularly in the helical segments of the (αα\textsubscript{2}) barrel structure (Fig. 3, A and B). Pairwise superimposition of the Cα atoms resulted in an average pairwise r.m.s. deviation of 1.0 Å among all of the structures (range 0.7–1.1 Å; Table II), with a greater conservation within the helices at the core of the barrel (Fig. 3B). Extending outward through the core of the barrel away from the β hairpin is a broadened funnel-shaped opening. Residues at the exterior of this opening comprise a complex series of loop and β-sheet structures (Fig. 2, LC side). Structural superimposition of the Class 1 mannosidases indicates that variation in amino acid sequence and structure is considerably greater in this region (Fig. 3B).

There is also significantly less conservation in sequence and structure at the NH\textsubscript{2}-terminal and COOH-terminal extensions beyond the (αα\textsubscript{2}) barrel and COOH-terminal hairpin structures. The Golgi mannosidase IA structure reveals a 14-amino acid sequence at the NH\textsubscript{2} terminus that lies across the SC side of the barrel. The lack of sequence conservation with other Class 1 mannosidases, the lack of an ordered secondary structure for this sequence, and the fact that prior isolations of this enzyme contained a smaller and more heterogeneous NH\textsubscript{2}-terminal extension (4–6 amino acids) based on Edman sequencing, would indicate that this region is not essential for catalytic activity or enzyme stability. At the COOH terminus, the extension beyond the β-hairpin structure is only three amino acids for murine Golgi mannosidase IA, similar to the T. reesei (29) and P. citrinum (30) α-mannosidases and human ER mannosidase I (25) (2, 3, and 3 amino acids, respectively) but considerably smaller than the COOH-terminal extension in the S. cerevisiae ER mannosidase I (17 amino acids) (27). It is not clear whether the former enzymes retain further COOH-terminal peptide sequences that are not ordered in the crystal lattice or whether proteolysis resulted in truncation of the respective peptides.

Proposed Catalytic Residues and Conserved Structures among the Class 1 Mannosidases—Prior studies on ER mannosidase I from human (Protein Data Bank codes 1FO2 and 1FO3 (25)) and S. cerevisiae (Protein Data Bank code 1G61 (49)) sources demonstrated that the inhibitors dMNJ and kifunensine bind to the interior of the barrel structure at the presumed -1 glycone binding site. This binding results in the interaction between the O-2’ and O-3’ hydroxyls of the inhibitor with the protein-bound calcium ion and a combination of hydrogen bonding and van der Waals interactions to result in an unusual C4 sugar conformation for the glycone in the -1 binding site. Attempts to co-crystallize or soak preformed crystals of Golgi mannosidase IA with dMNJ were unsuccessful. No appreciable electron density was present in the -1 binding site.
Alignment of Class 1 mannosidase catalytic domain protein sequences. The sequence of the catalytic domain of murine Golgi mannosidase IA is compared with the catalytic domain sequences of other Class 1 mannosidases for which structural data are available (A). Sequence data include human ER mannosidase I (HsERManI; Protein Data Bank 1FO2 (25)), S. cerevisiae ER mannosidase I (ScERManI; Protein Data Bank 1DL2 (27)), P. citrinum α-mannosidase (PcManI; Protein Data Bank 1KKT (80)), T. reesei α-mannosidase (TrManI; Protein Data Bank 1HCU (29)), mouse Golgi mannosidase IA (MmGManIA; Protein Data Bank 1NXC, this work). Sequences are numbered from the NH₂ terminus of the intact protein. The coloring of the sequence labels are the same as the coloring of the Cα backbone and carbon atom coloring in B and C, respectively. Sequences were aligned using ClustalW (41), and A was prepared using ESPript (46). Colored helical segments above or below the sequence alignment correspond to the similarly colored helical segments in Fig. 2. The arrows indicate segments of β-sheet structure. Secondary structure is shown for human ER mannosidase I (above the sequence) and mouse Golgi mannosidase IA (below the sequence). Green Asn residues with an asterisk in the sequence alignments are sites of N-glycosylation within the respective protein in the crystal structure. B, a stereo view of the aligned Cα backbone representation of mouse Golgi mannosidase IA (MmGManIA, orange), human ER mannosidase I (HsERManI, magenta), and P. citrinum α-mannosidase (PcManI, green), demonstrating the similarity in backbone position in the core of the barrel but deviation in structure in the periphery of the barrel. C, a stereo view of the positions of amino acid side chains in the −1 glycone binding site for the aligned Class I mannosidases (within 5 Å of dMNJ in the aligned structures). Protein structures were aligned for mouse Golgi mannosidase IA (orange), human ER mannosidase I (magenta), S. cerevisiae ER mannosidase I (cyan), P. citrinum α-mannosidase (green), and the T. reesei α-mannosidase (gray), and the amino acid side chains are shown in stick form and the associated calcium ion is shown in a space-filling representation in the color of the respective protein structure. The structure of dMNJ in the −1 glycone binding site was derived from the human ER mannosidase I structure (Protein Data Bank 1PO2 (25)) and is shown in white stick form. Residue numbering is based on the residues from mouse Golgi mannosidase IA.
either as a consequence of insufficient inhibitor in the crystallization solution or as a result of competition between inhibitor binding and glycan binding to the enzyme (see below). Despite the absence of the inhibitor in the $\alpha$-1 binding site, comparison of the structure with the other family 47 mannosidases indicated that there is a high degree of conservation in sequence and amino acid side chain position within the $\alpha$-1 mannose binding site (Fig. 3, A and C). Pairwise comparison of the positions of residues within the $\alpha$-1 binding site for all of the Class 1 enzymes (within 5 Å of dMNJ in the $\alpha$-1 binding site of human ER mannosidase I (Protein Data Bank code 1FO2)) indicated that the positions of these residues are highly conserved, with an average r.m.s. deviation for all of the residues in the binding site of 0.4 Å (range 0.3–0.5 Å; Table II and Fig. 3C). Thus, the interactions of the glycone mannose residue with the $\alpha$-1 binding site and the catalytic mechanism of action are likely to be conserved among all of the members of this enzyme family (25).

### Table II

|                | MnGManIA | HsERManI | PcManI | TrManI | ScERManI |
|----------------|----------|----------|--------|--------|----------|
| MmGManIA       | 1.1 Å    | 1.0 Å    | 1.1 Å  | 1.1 Å  | 1.1 Å    |
| (418 res.)     | (391 res.) | (396 res.) | (395 res.) | (409 res.) |
| MmGManIA       | 0.4 Å    | 0.3 Å    | 0.4 Å  | 0.4 Å  | 0.3 Å    |
| (124 atoms)    | (124 atoms) | (124 atoms) | (124 atoms) | (124 atoms) |
| HsERManI       | 1.1 Å    | 1.0 Å    | 0.7 Å  | 0.7 Å  | 0.5 Å    |
| (418 res.)     | (391 res.) | (396 res.) | (407 res.) | (409 res.) |
| PcManI         | 0.9 Å    | 0.8 Å    | 1.1 Å  | 1.1 Å  | 1.1 Å    |
| (407 res.)     | (406 res.) | (407 res.) | (389 res.) | (387 res.) |
| TrManI         | 1.0 Å    | 0.8 Å    | 0.5 Å  | 0.5 Å  | 0.5 Å    |
| (456 res.)     | (456 res.) | (456 res.) | (456 res.) | (456 res.) |
| ScERManI       | 1.1 Å    | 0.9 Å    | 1.1 Å  | 1.1 Å  | 1.1 Å    |
| (407 res.)     | (407 res.) | (407 res.) | (389 res.) | (387 res.) |

**FIG. 3—continued**

The protein structures for five Class 1 mannosidases (human ER mannosidase I, HsERManI, Protein Data Bank 1FO2 (25); *P. citrinum* α1,2-mannosidase, PcManI, Protein Data Bank 1KKT (30); *T. reesei* α1,2-mannosidase, TrManI, Protein Data Bank 1HCU (29); *S. cerevisiae* ER mannosidase I, ScERManI, Protein Data Bank 1DL2 (27); and murine Golgi mannosidase IA, MmGManIA, Protein Data Bank 1NXC (this work)) were aligned using the DeepView/Swiss-PDB viewer (40). The lower left cells in the table show the r.m.s. deviation for the pairwise comparison of the Cα backbone residues between the respective full structures. The number in parenthesis reflects the number of residues (res.) used in the r.m.s. calculation. The upper right cells show the r.m.s. deviation for the pairwise comparison of the backbone and side chain residues in the core of the glycone (−1) binding site of the respective structures (residues within 5 Å of the dMNJ molecule in the 1FO2 structure (25)). The number in parenthesis reflects the number of atoms used in the r.m.s. calculation.
High Mannose Oligosaccharide-Protein Interactions—The Golgi mannosidase IA coding region contains a single consensus Asn-linked glycosylation site at Asn153 (31) (Fig. 3A). This enzyme expressed in Pichia contains predominately Man5GlcNAc2 and to a lesser degree Man6GlcNAc2 structures (approximate ratio 2:1; data not shown). Glycosylation also became evident during inspection of an Fo – Fc map (Fig. 4), where an oligosaccharide with two N-acetylglucosamine and five mannose residues was clearly visible. This structure represents the normal oligosaccharide limit-digestion product for Golgi mannosidase IA (23) (Fig. 1C). Since an oligosaccharide structure cleaved beyond Man5GlcNAc2 is not normally found in P. pastoris (50, 51), these data indicate that the recombinant enzyme has probably trimmed the mannose residues on its own cohort of N-glycans either during expression, purification, or crystallization. A weak electron density for an α1,6-mannose residue was also found linked to mannose residue M5, indicating that the Man5GlcNAc2 glycan structure resulted from the extension of an α1,6-mannan backbone at this position, as is common in P. pastoris (50, 51). The lack of a complete electron density at this site probably results from the heterogeneity of glycosylation at this site and possible disorder resulting from a greater flexibility of this residue in the solvent.

Resolution of Asn-linked glycan structures by x-ray diffraction beyond the core GlcNAc residues is rare unless the distal residues are constrained by interactions within the crystal lattice. Inspection of the normalized Fo – Fc map indicated that the majority of the glycan structure had a low temperature factor as a result of interactions with the top of the funnel-shaped pocket of an adjoining protein monomer in the crystal lattice (Fig. 2). The matrix of interactions stabilizing this conformation included direct and through-water hydrogen bonds as well as hydrophobic interactions with the substrate binding cleft in the adjoining molecule (Fig. 5, A and B). The position of the oligosaccharide in the binding site suggested that the complex reflects an enzyme-product complex, since residue M6 was in the position proposed for the +1 substrate binding site and a nascent glycan structure would position the M6-α1,2-M9 residues within the +1 and −1 binding sites. The nature of the complex is superficially similar to the complex between a Man5GlcNAc2 glycan and the active site of S. cerevisiae ER mannosidase I (27). In the latter structure, the enzyme product complex in the crystal lattice results in the insertion of residue M7 into the +1 binding site, representing an enzyme product complex where the M7-α1,2-M10 linkage would bridge the +1 and −1 binding sites. The position and interactions between the M6 residue in the +1 binding site of Golgi mannosidase IA (Fig. 6, A and C) and M7 in the equivalent site in the S. cerevisiae ER mannosidase I (Fig. 6, B and D) can be virtually superimposed. Most notably, the conserved interactions include a pair of hydrogen bonds between Asp115 (Asp775 in yeast ER mannosidase I) and mannose residue M6 (hydrogen bonds between carboxyl O-2’ and the O-3’ hydroxyl as well as the carboxyl O-α1 and the O-4’ hydroxyl) (Fig. 5, A and B). Further interactions include a hydrogen bond between the peptide bond nitrogen of Leu413 (Arg273 in the yeast enzyme) and the M6 O-4’ hydroxyl and a hydrogen bond between the carboxyl O-α1 of Glu335 (Glu197 in the yeast enzyme) and the M6 O-6’ hydroxyl. Indirect interactions are found between the O-2’ of Glu332 (Glu192 in the yeast enzyme) and the O-2’ hydroxyl and the O-5 of M6 through two water molecules (HOH1058 and HOH1012; Fig. 5, A and B). Similarly, the position of the proposed catalytic proton donor, Glu282 (Glu132 in the yeast enzyme), in the unusual water molecule-mediated (HOH1058) protonation mechanism is conserved in position, as are the catalytic base, Glu349 (Glu435 in the yeast enzyme), and the water nucleophile (HOH1089) (25). Thus, the positioning of the presumed Man-α1,2-Man substrate linkage in the +1 and −1 sites employs functionally identical residues, and catalysis is presumably accomplished by functionally identical carboxyl side chains and water molecules in the two enzymes.

In contrast to the similarities in binding of the oligosaccharide to the +1 site, binding interactions in the +2 and distal sites are quite distinct between murine Golgi mannosidase I and S. cerevisiae ER mannosidase I. The majority of the interactions between the oligosaccharide and the ≥ +2 binding sites for the Golgi enzyme involve nine indirect hydrogen bonds through bridging water molecules (Fig. 5, A and B). Direct interactions are limited to hydrogen bonds between the O-2’ hydroxyl of M4 and carboxyl O-ε2 of Glu524, as well as an interaction between the O-3’ hydroxyl of M5 and the Oγ of Ser253. In addition, there is a direct stacking of Trp441 over the flattened sugar ring structure of the core NAG2 (average distance 3.9 Å) in an apparent hydrophobic interaction. Other limited interactions occur within the crystal lattice between the oligosaccharide and the protein within the same molecule.
green side chains in Fig. 5, A and B). Thus, the positioning of the oligosaccharide in the binding cleft is facilitated by an array of hydrogen bonding interactions predominately anchored by the extensive interactions at the +1 site and the hydrophobic interactions at the core of the glycan.

The oligosaccharide conformation is contrasted with the con-

FIG. 5. Interaction of mouse Golgi mannosidase IA with residues in the active site. A, a schematic diagram of the interacting residues between the binding pocket of Golgi mannosidase IA and the bound glycan enzymatic product. The coloring of glycan residues is the same as in Fig. 1. Indirect hydrogen bonds through intervening water molecules are indicated by black dotted lines. Direct hydrogen bonds between the protein and the bound glycan are indicated by red dotted lines. The hydrophobic stacking between Trp341 and NAG2 is indicated by the broad cyan dotted lines. Residues labeled with green amino acid numbering are residues that are derived from the same protein unit as the attached glycan. The monosaccharide shown in gray is an α1,6-mannose added to the trimmed oligosaccharide during secretion in P. pastoris. B, stereo view of the oligosaccharide in stick form with a display of the indirect hydrogen bonding interactions shown as green dotted lines and direct hydrogen bonds shown as red dotted lines. The coloring of the glycan carbon atoms follows the same pattern as in Figs. 1 and 5A. Only the water molecules that are indirectly associated with a hydrogen bonding interaction between the oligosaccharide and the protein are shown. Amino acid side chain carbon atoms shown in white are in the glycan binding site. Amino acid side chains in green are from the same protein unit as the attached glycan.
formation of the glycan in *S. cerevisiae* ER mannosidase I, where the dominant interactions in the ±2 binding sites are five hydrogen bonds from Arg273 to residues M7, M4, and M3 (27, 28). Additional direct hydrogen bonds between the protein and residues M5, M4, and M6 stabilize the conformation of the oligosaccharide in the cleft distal to the +1 site. Whereas the core GlcNAc residues extend in approximately the same direction between the ER and Golgi mannosidases, the major difference in oligosaccharide conformation between the ER and Golgi enzymes is a rotation around the Man-α1,6-Man linkage extending between residues M3 and M4 to present a different branch into the +1 and −1 sites (Fig. 6).

**Topography of the Binding Cleft between the Class 1 Mannosidases**—A comparison of the binding site topologies among known family 47 α-mannosidases, including Golgi mannosidase IA, indicates that the +1 and −1 binding sites in the core of the barrel structure are almost identical among the five Class 1 mannosidases (25, 29, 30, 32). As predicted from the conserved positions of the residues in this region (Fig. 3C), the diameters of the +1 binding sites are quite similar among the Class 1 mannosidases (Fig. 7, A–F, lower distance measurements). Extending outward from the constricted pocket containing the +1 and −1 binding sites is an extended cleft containing binding sites for ±2 residues (Fig. 7, A–F). The dimensions of this cleft vary significantly among the Class 1 mannosidases. The glycan binding clefts for human and *S. cerevisiae* ER mannosidase I are narrow in one dimension (i.e. 17.6 Å representative dimension for human ER mannosidase I) (Fig. 7F) and broad in the other dimension (i.e. 33.1 Å representative dimension for human ER mannosidase I) (Fig. 7E) to accommodate the extended branches of the flattened oligosaccharide substrate (25, 32).

Protein-glycan interactions for *S. cerevisiae* ER mannosidase I extend across the base and up the sides of the cleft, including interactions with all of the mannose residues up to the glycosidic oxygen between residue M3 and NAG2 (27). By contrast, the equivalent oligosaccharide binding sites of the broad specificity α-mannosidases from *P. citrinum* and *T. reesei* have a very broad cleft beyond the +1 binding site (i.e. 22.1 × 36.7 Å representative dimensions for *P. citrinum* α-mannosidase) (Fig. 7, C and D) and are unlikely to provide direct interactions with an oligosaccharide substrate beyond those residues that are in direct contact with the base of the cleft adjacent to the catalytic pocket (29, 30). Unlike the broad specificity enzymes from the filamentous fungi, Golgi mannosidase IA has a constricted substrate binding cleft (i.e. 14.5 × 22.3 Å representative dimensions) (Fig. 7, A and B), considerably narrower in each dimension than even the ER mannosidases. The positioning of the oligosaccharide in the narrow binding pocket extends direct and indirect interactions throughout the oligosaccharide up to and including a hydrophobic stacking with the core GlcNAc residue (NAG2) through the matrix of hydrogen bonding interactions described above.

**Oligosaccharide Conformation Comparison in the Binding Sites of Class 1 α-Mannosidases**—The recently published structures of α-mannosidases from the filamentous fungi indicate that a broad and spacious oligosaccharide binding site adjacent to the +1 and −1 sites could accommodate the insertion of a wide range of oligosaccharide branches into the catalytic pockets of these enzymes (29, 30). These conclusions were supported by modeling studies indicating that only a restricted set of oligosaccharide structures and conformations were allowed in the binding sites of yeast and human ER mannosidase I, whereas a greater number of oligosaccharide conformations and branches could be accommodated by the broad specificity enzyme from *P. citrinum* (30). A pairwise comparison was made between the oligosaccharide conformation associated with the Golgi mannosidase IA binding cleft versus the oligosaccharide associated with the *S. cerevisiae* ER mannosidase I binding site (Table III) and the two most favorable oligosaccharide conformations modeled into the binding site of human ER mannosidase I containing residue M6 in the +1 site (branch C in Table II of Ref. 30). Oligosaccharide alignment was performed using the residue in the +1 site as a reference (M6 for Golgi mannosidase IA and human ER mannosidase I and M7 for yeast enzyme), and pairwise r.m.s. distance measurements and glycosidic torsion angles were calculated for corresponding residues in the oligosaccharide structures (Tables III and IV). These comparisons indicated that neither *S. cerevisiae* ER mannosidase I oligosaccharide nor the favored models associated with human ER mannosidase I (30) had a high degree of overall similarity to the oligosaccharide conformation of the Golgi enzyme. In model 2 of human ER mannosidase I (30),
residues M3 and M5 were in a similar location (r.m.s. distances <2 Å, Table III), but other residues including the +2 residue (M4) were in significantly different positions (r.m.s. distances of >4.5 Å from the corresponding residue in the Golgi enzyme).

In contrast, some of the most favored oligosaccharide conformations modeled into the binding site of the *P. citrinum* α1,2-mannosidase (30) had a reasonable degree of similarity to the corresponding conformation of the oligosaccharide associated

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**Fig. 7. Cross-section views of the active sites and glycan binding clefts of representative Class 1 mannosidases.** Three representative Class 1 α-mannosidase structures (mouse Golgi mannosidase IA (A and B), *P. citrinum* α-mannosidase (C and D), and human ER mannosidase I (E and F)) were aligned as in Fig. 3B, and residues within 8 Å of the glycan associated with the mouse Golgi mannosidase IA oligosaccharide binding site were selected for surface rendering. Two cross-sectioned enzyme surface representations were rendered at right angles from each other for each of the enzymes (gray surface). For reference, the glycan from the mouse Golgi mannosidase IA structure is shown in yellow stick form in each of the respective enzyme surface structures. dMNJ bound in the +1 glycone site (green stick form) and the calcium ion (blue space-filling representation) bound at the base of the active site pocket are also shown and were obtained from the human ER mannosidase I structure (Protein Data Bank 1F02 (25)). Measurements across the +1 binding site were made using the nearest equivalent atoms spanning each of the respective cross-sections (lower indicated angstrom measurements in each respective panel). Similarly, measurements were made across the upper portion of the glycan binding clefts at approximately similar positions and orientations (upper angstrom measurements in each respective panel). Significant differences in the structures and residues flanking the glycan binding clefts in each of the enzymes made it impossible to measure identical spans for each enzyme, but the residues used in the respective measurements are indicated and shown in stick form in each panel. A, C, and E are all in identical orientations, and B, D, and F are all in identical orientations. ER Man I, ER mannosidase I; Golgi Man I, Golgi mannosidase I.
with the Golgi mannosidase IA binding site (Tables III and IV). In particular, when residue M6 was used as the reference point for the pairwise oligosaccharide alignment, P. citrinum model 3 positioned the most proximal residues (M4, M7, and M3) within r.m.s. distances of 1.0, 2.0, and 2.6 Å, respectively, from the corresponding residues in the Golgi enzyme. These residues are predominately associated with the base of the cleft adjacent to the active site pocket in Golgi mannosidase IA. Other residues in this oligosaccharide are considerably further away from the conformation of the corresponding residues in the Golgi enzyme, indicating that the similarity is restricted to the residues most proximal to the catalytic site. Thus, despite the differences in the binding site topologies between murine Golgi mannosidase I and the P. citrinum α1,2-mannosidase, the active sites of these two enzymes can accommodate similar oligosaccharide conformations of the adjacent residues during the cleavage of the M6-α1,2-M9 linkage.

**DISCUSSION**

We present here the structure of the soluble catalytic domain of mouse Golgi mannosidase IA, an enzyme involved in the processing of high mannose N-glycans to Man₉GlcNAc₂ structures as a key step in the maturation of glycoproteins to complex-type oligosaccharides on cellular and secreted glycoproteins (1). The structure of the Golgi enzyme was solved by molecular replacement, revealing an (ααα)₆ barrel with a high degree of similarity to the other four known Class 1 mannosidase structures (25, 27, 29, 30), particularly in the helical segments and the core of the barrel near catalytic site. Both S. cerevisiae ER mannosidase I and Golgi mannosidase IA have been shown to be inverting glycosidases (23, 52), and hypotheses have been proposed for a nonstandard inverting mechanism involving a through-water protonation of the glycosidic oxygen (25) and the formation of an unusual 1C₄ sugar conformation for the glycone. Similarities in structure around the +1 binding site among all of the Class 1 mannosidases would indicate that all of the members of this family have the same active site chemistry and mechanism of action. In contrast to similarities in their overall structures and catalytic mechanisms, the members of the various Class 1 mannosidases have considerable differences in high mannose oligosaccharide branch specificity (6). The branch specificity for ER mannosidase I was shown to be at least partially determined by the extensive interactions between Arg⁷⁷³ to Leu resulted in a lower efficiency and broader substrate specificity for the enzyme to result in a hybrid activity between ER mannosidase I (residue M10 was still cleaved first) and the Golgi mannosidases (digestion proceeded to Man₉GlcNAc₂).

**TABLE III**

| r.m.s. distance measurements between oligosaccharide residues in the Golgi mannosidase IA substrate binding site and equivalent residues in other oligosaccharide structures |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | NAG1 | NAG2 | M3  | M4  | M5  | M6  | M7  |
| ScERManI        | 4.5  | 2.9  | 4.4 | 4.1 | 9.0 | 0.0* | 4.2* |
| HsERManI: model 1 | 18.9 | 9.3  | 4.1 | 4.6 | 10.7| 0.0 | 11.5|
| HsERManI: model 2 | 4.1  | 3.3  | 2.0 | 4.6 | 2.0 | 0.0 | 11.4|
| PcManI: model 1  | 8.5  | 3.8  | 2.2 | 1.1 | 4.7 | 0.0 | 2.5 |
| PcManI: model 2  | 13.2 | 8.5  | 3.0 | 2.4 | 3.5 | 0.0 | 5.7 |
| PcManI: model 3  | 13.0 | 8.0  | 2.6 | 1.0 | 7.3 | 0.0 | 2.0 |
| PcManI: model 4  | 11.1 | 6.0  | 5.2 | 2.5 | 10.7| 0.0 | 5.9 |

**TABLE IV**

Comparison of oligosaccharides torsion angles (degrees) from crystal structures and conformational modeling

Torsion angles are shown for the oligosaccharides found in the respective crystal structures for the murine Golgi mannosidase IA (MmGManI) (this work) and S. cerevisiae ER mannosidase I (ScERManI) (27). Additionally, the torsion angles of favored oligosaccharide conformations modeled into the active sites of human ER mannosidase I (HsERManI) (two models) and the P. citrinum α1,2-mannosidase (PcManI) (four models) is shown where the M6 residue is in the +1 binding site (branch C in Table II of Ref. 30).
The presence of a Leu residue in the equivalent position (Leu\textsuperscript{279}) in Golgi mannosidase I\textsubscript{A} is not sufficient to provide this hybrid specificity in the latter subfamily of enzymes, since this enzyme cleaves the M10 residue at least 10-fold more slowly than the other Man-α1,2-Man linkages in the substrate (23).

Glycan recognition by Golgi mannosidase I\textsubscript{A} has been examined here through the characterization of a presumed enzyme-product complex trapped in the crystal structure. This structure provides a basis for comparison with the structure of ER mannosidase I containing a similar type of enzyme-product complex. Both enzymes have a collection of interactions between the oligosaccharide binding cleft and the extended glycan structure. Interactions with the +1 mannose residue are most extensive and act to position the respective Man-α1,2-Man linkage adjacent to the catalytic residues and position the glycone deep into the active site pocket, where it can interact with the conserved Ca\textsuperscript{2+} ion and the other critical residues in the –1 binding site. Further out in the oligosaccharide binding cleft, beyond the +1 residue, the two enzymes diverge in sequence and structure. ER mannosidase I has more extensive direct interactions with the glycan structure, particularly through interactions with Arg\textsuperscript{273} and additional direct interactions with other side chains in the binding cleft (27). Golgi mannosidase I\textsubscript{A} has an array of interactions that restrict the mobility of the glycan within the narrow binding cleft, but many of the interactions with the glycan beyond the +1 residue are indirect, though-water interactions and a hydrophobic stacking interaction between Trp\textsuperscript{441} and a NAG residue in the glycan core. Whereas there are significant differences in the positions of individual sugars in the binding pockets of the ER and Golgi enzymes, the main difference between the two bound glycan structures is the 180° rotation of glycan around the M3-α1,6-M4 linkage to reverse the positions of residues M6 and M7. These differences alone can account for the distinctions in branch specificity between the two enzymes, allowing ER mannosidase I to cleave residue M10 while Golgi mannosidase I\textsubscript{A} cleaves residue M9. The ordered and sequential nature of oligosaccharide cleavage of Man\textsubscript{9-6GlCNAc} to Man\textsubscript{9-3GlCNAc} by Golgi mannosidase I\textsubscript{A} (23) would strongly suggest that the narrow cleft and the array of indirect interactions are appropriately positioned for the selective insertion of multiple individual manno branches into the active site pocket for glycoside bond hydrolysis.

Added support for an interaction between Golgi mannosidase I\textsubscript{A} and the core NAG residues in the glycan substrate comes from prior data from Bause et al. (53), who compared the cleavage of Man\textsubscript{9-6GlcNAc} glycopeptides or free Man\textsubscript{9-6GlcNAc} oligosaccharides versus reduced Man\textsubscript{9-6GlcNAc\textsubscript{2-ol}} oligosaccharides by the porcine Golgi mannosidase I (previously termed Man9-mannosidase (53)). Reduction of the free oligosaccharides altered the ability of enzyme to cleave residue M10, indicating that interactions between the closed acetal ring structures of the core NAG residues in the oligosaccharide substrate influenced the binding and cleavage of distal residues in the glycan substrates. The presence of a loop sequence containing the critical Trp\textsuperscript{441} residue that is uniquely conserved among all of the mammalian Golgi mannosidase subfamily members (24, 31, 54) also supports a role for hydrophobic stacking interactions with the core NAG residue during substrate recognition.

Previous studies have suggested that the broader substrate specificities of the Class I mannosidases from filamentous fungi (\textit{T. reeset} and \textit{P. citrinum}) result from a broad pocket adjacent to the +1 and –1 binding sites, in contrast to the narrow clefts for the human and yeast ER mannosidase I structures (29, 30) and the even more constricted binding cleft for Golgi mannosidase I\textsubscript{A} described here. Comparison of the topologies among the Class I mannosidases (Fig. 7) adjacent to the +1 and –1 sites confirms that the broader binding clefts are unique for the \textit{T. reeset} and \textit{P. citrinum} enzymes. However, modeling of high mannose glycan structures into the binding cleft and active site of the \textit{P. citrinum} α-mannosidase (30) revealed structures that were similar to the conformations of the proximal glycan residues adjacent to the catalytic site for Golgi mannosidase I\textsubscript{A}. It is also striking that the substrate specificity and order of mannose removal for α-mannosidases from filamentous fungi, including \textit{P. citrinum}, is quite similar to Golgi mannosidase I\textsubscript{A} (55, 56). These data suggest that the glycan interactions adjacent to the active site pocket in both enzymes may be sufficient to provide the observed order of glycan digestion for these broad specificity enzymes.

The identification of enzyme product complexes in the crystal structures of both the ER and Golgi mannosidases suggests that the enzymes have a strong affinity for binding of their respective oligosaccharide enzymatic products. In support of this hypothesis, we have recently obtained binding data by surface plasmon resonance indicating that high mannose glycans can directly interact with the ER and Golgi mannosidase binding clefts with micromolar affinities irrespective of glycosidase activity in the –1 glycone binding site.\textsuperscript{2} These data also indicate that the homologous and nonhydrolytic EDEM subfamily of proteins could accomplish their presumed glycan-lectin interactions (8, 9, 13, 16, 57) during ER-associated degradation of glycoproteins by taking advantage of the binding site cleft without the need for invoking the insertion of the glycan structure into the –1 glycone binding site. Future studies will probably identify the distinctions between the determinants required for selective glycan isomer binding and hydrolysis by the ER and Golgi mannosidases, whereas the EDEM family of lectins presumably employ a similar structural fold for glycan binding without the requirement for glycoside bond hydrolysis. The manner in which these Class I mannosidases accomplish their varied roles in glycan maturation and ER-associated degradation through the use of a common structural fold employed for high mannose glycan recognition and catalysis will present a paradigm for studies of glycosidase and lectin interactions in the future.

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REFERENCES

1. Moremen, K. (2001) in Oligosaccharides in Chemistry and Biology: A Comprehensive Handbook (Ernst, B., Hart, G., and Sinay, P., eds) Vol. II, pp. 81–117, John Wiley and Sons, Inc., New York.
2. Kornfeld, R., and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664.
3. Herscovics, A. (1999) Biochim. Biophys. Acta 1426, 275–285.
4. Herscovics, A. (1999) in Comprehensive Natural Products Chemistry (Pinto, B. M., ed) Vol. 3, pp. 13–35, Elsevier, New York.
5. Herscovics, A. (1999) Biochim. Biophys. Acta 1473, 96–107.
6. Moremen, K. W., Troumle, R. B., and Herscovics, A. (1984) Glycobiology 4, 113–125.
7. Daniel, P. F., Winchester, B., and Warren, C. D. (1994) Glycobiology 4, 551–568.
8. Oda, Y., Hosokawa, N., Wada, I., and Nagata, K. (2003) Science 299, 1394–1397.
9. Hosokawa, N., Wada, I., Hasegawa, K., Yuzhiru, T., Tremblay, L. O., Hersco- vics, A., and Nagata, K. (2001) EMBO Rep. 2, 415–422.
10. Nakatsukasa, K., Nishikawa, S., Hosokawa, N., Nagata, K., and Endo, T. (2001) J. Biol. Chem. 276, 8665–8668.
11. Jakob, C. A., Bodmer, D., Sprirg, U., Battig, P., Marcell, A., Dignard, D., Bergeron, J. J., Thomas, D. Y., and Aebi, M. (2001) EMBO Rep. 2, 423–430.
12. Wang, T., and Hebert, D. N. (2003) Nat. Struct. Biol. 10, 319–321.

\textsuperscript{2}K. Karaveg and K. W. Moremen, manuscript in preparation.
Structure of Mouse Golgi α-Mannosidase IA Reveals the Molecular Basis for Substrate Specificity among Class 1 (Family 47 Glycosylhydrolase) α-1,2-Mannosidases

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