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The Structure of HLA-DM, the Peptide Exchange Catalyst that Loads Antigen onto Class II MHC Molecules during Antigen Presentation

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Summary

The three-dimensional structure of the soluble ectodomain of HLA-DM has been determined to 2.5 Å resolution by X-ray crystallography. HLA-DM has both peptide exchange activity and acts as a chaperone to peptide-free class II MHC molecules. As predicted, the structure is similar to that of classical class II MHC molecules except that the peptide-binding site is altered to an almost fully closed groove. An unusual cavity is found at the center of the region that binds peptides in class II MHC molecules, and a tryptophan-rich lateral surface is identified that is a candidate both for binding to HLA-DR, to effect catalysis, and to HLA-DO, an inhibitor.

Introduction

After biosynthesis into the endoplasmic reticulum, class II MHC molecules bind to the invariant chain protein (Ii) and are escorted to an endosomal compartment. There proteolysis removes Ii and HLA-DM catalyses exchange of the contents of the MHC-binding site, a peptide remnant of Ii named CLIP, for antigenic peptides (Riberdy et al., 1992; Sette et al., 1992; Cresswell, 1994, 1996; Denzin and Cresswell, 1995; Sherman et al., 1995; Sloan et al., 1995; Wolf and Ploegh, 1995). Leaving DM behind in endosomes, peptide/MHC complexes move to the cell surface with the peptides kinetically trapped (dissociation $t_{1/2} > 50$ hr), a requirement as extracellular antigenic peptide concentrations are vanishingly small. Mutant cells lacking DM express class II molecules on the cell surface loaded with CLIP (Fing et al., 1994; Morris et al., 1994), which occupies the peptide-binding site like an antigenic peptide but with nonoptimal anchoring residues (Ghosh et al., 1995).

In vitro, tight peptide binding (Watts and McConnell, 1986; Buus et al., 1987, 1988), even to peptide-free, "empty" class II molecules, is very slow ($t_{1/2}$ = 24 hr) (Stern and Wiley, 1992); DM increases this rate by $\sim 10^4$ (Weber et al., 1996). It binds to class II molecules, like HLA-DR, acting as a chaperone by preventing the aggregation of empty DR (Stern and Wiley, 1992; Germain and Rinker, 1993), which increases the yield of peptide complexes (Denzin et al., 1996; Sanderson et al., 1996; Kropshofer et al., 1997) as observed earlier for the DR-binding molecules SEB and a trimeric domain of Ii (Park et al., 1995). It also acts as an exchange enzyme, increasing the rate of peptide binding, apparently by stabilizing a strained, transition-state conformation of DR that is "open," thus favoring the dissociation and association of peptides (Ghosh et al., 1995; Denzin et al., 1996; Sanderson et al., 1996; Vogt et al., 1996; Weber et al., 1996; Kropshofer et al., 1997). The crystal structure of DM, reported here, reveals a class II–like molecule without a peptide-binding groove but containing a curious polar, possibly vestigial, pocket and a tryptophan-rich lateral surface that is a candidate for binding the inhibitor HLA-DO (Liljedahl et al., 1996; Denzin et al., 1997; van Ham et al., 1997) and if bound to substrates like HLA-DR might distort the peptide-binding site.

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Results

Structure Determination

HLA-DM with peptide epitope tags ("FLAG" on α and "KT3" on β) replacing the transmembrane and cytoplasmic regions of the α and β polypeptides was produced in *Drosophila* S2 cells and purified as previously described (Sloan et al., 1995) (see Experimental Procedures). Crystals were grown near the pH optimum (pH 5.4) of the enzyme (see Experimental Procedures), and the crystalline protein was shown by a native gel-shift assay to enhance peptide loading of both empty and CLIP-containing, soluble HLA-DR1 and HLA-DR4 (Experimental Procedures; data not shown). The structure was determined by molecular replacement, with HLA-DR1 (Stern et al., 1994) as a search model, and refined to 2.5 Å resolution (R_{free} = .256, R_{work} = .194) (Experimental Procedures). The final model contains α chain residues 13-199 and β 3-192, 198 solvent molecules, and three saccharides attached at Asn α15.

Three-Dimensional Structure of HLA-DM Compared to HLA-DR

The overall fold and domain organization of DM is similar to that of classical class II MHC molecules (Busch et al., 1998b; Dessen et al., 1997; references therein), as expected from the 28% sequence identity (Cho et al., 1991; Kelly et al., 1991) and from data indicating the requirement for formation of a homologous disulfide bond (Busch et al., 1998a) (rmsd = 2.9 Å for 360 Cα pairs) (Figure 1A). DM contains two novel disulfide bonds (α 24-79 and β 25-35) (green, Figure 1A) in the region homologous to the peptide-binding site of DR. None of the conserved residues identified in DR1 that form hydrogen bonds to the main chain atoms of bound peptides are conserved in DM and none of the pockets identified for binding the side chains of peptides are present (Stern et al., 1994) (albeit, see below). Instead of forming a peptide-binding groove, the α helices of the α1 and β1 domains of DM contact each other over the first and last thirds of their lengths (Figure 1B), like those of the class I-like, neonatal Fc receptor (FcRn)

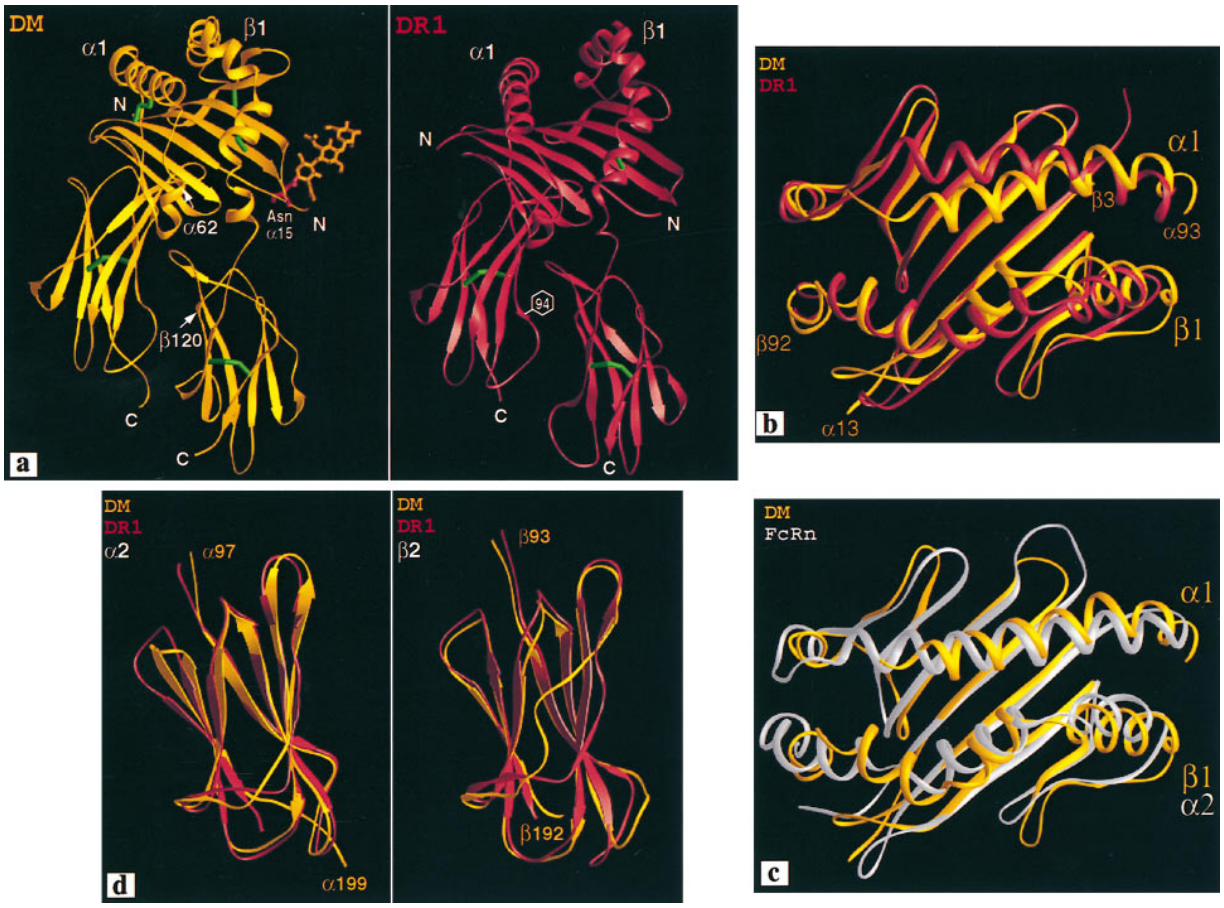

Figure 1. Comparison of the Structures of HLA-DM, HLA-DR1, and FcRn, the Neonatal Fc Receptor

(A) DM (yellow), DR1 (red), disulfide bonds (green). The locations of tryptophans α62 and β120 are labeled with arrows.
(B) Superposition of the α1 and β1 domains of HLA-DM (yellow) and HLA-DR1 (red). (rmsd = 2.5 Å for 160 Cα pairs).
(C) Superposition of the α1 and β1 domains of HLA-DM (yellow) and α1 and α2 domains of FcRn (gray) (rmsd = 2.4 Å for 153 Cα pairs).
(D) Superposition of the α2 and the β2 domains of HLA-DM (yellow) and HLA-DR1 (red) (α2 domain, rmsd = 1.04 Å for 92 Cα pairs; β2 domain, rmsd = 2.1 Å for 93 Cα pairs).

(Burmeister et al., 1994) (Figure 1C), precluding peptide binding as expected (Busch and Mellins, 1996). There are some differences in the conformations of several loops in the α2 and β2 domains of DM relative to DR (Figure 1D), but most are at crystal contacts that may distort their shapes. The β2 chain in DM, as it passes from the second β sheet back to the first, also takes a slightly different path than in DR (Figure 1D). These differences permit the formation of a dimer of DM heterodimers in the crystal, different from that that DR1 can form (see below) (Brown et al., 1993).

A Closed Groove

Three protuberant kinks in the β1 domain α helix cause a zigzag in its course across the domain (dashed line, Figure 2A). This separates the residues packed between the two domains into three apparent clusters (inserts in Figure 2). Two of the clusters, at the two ends of the domains, are nonpolar residues mediating tight packing (9.5 Å helix to helix) between the α helices of the α1 and β1 domains (Figure 2). The central cluster, by contrast, is polar, and charged residues (Figure 2A) form a 10 Å wide and 10 Å deep pocket (Figure 2B). This pocket is larger than a vestigial dimple found in the same region of FcRn (Burmeister et al., 1994) but smaller than the groove and cavity in the class I–like molecule CD1 (Zeng et al., 1997), which apparently presents lipid and glycolipid mycobacterial antigens (Beckman et al., 1994). The DM pocket is completely unlike the long groove of pockets in a class II molecule (Figure 2C).

A Central Pocket

At the bottom of this central pocket is a puzzling arrangement of a familiar triad, Serine β9, Histidine α20, and Glutamic acid α35 (or Aspartic acid α68), arranged like the catalytic triad in serine proteases and esterases (Figure 2A) (Stryer, 1981). But, this triad could not act as a protease because there is no groove to bind a peptide, rather just a dead-end pocket as might form an acetylesterase (Wei et al., 1995) or binding site for an amidated carboxylate or acetylated amine (caption Figure 2B). Importantly, there are no polar residues forming an oxy-anion hole to stabilize a tetrahedral intermediate generated by a serine nucleophilic attack on a carbonyl. Thus, the juxtaposition of Ser/His/Glu (Asp), conserved in all known DM sequences, does not form

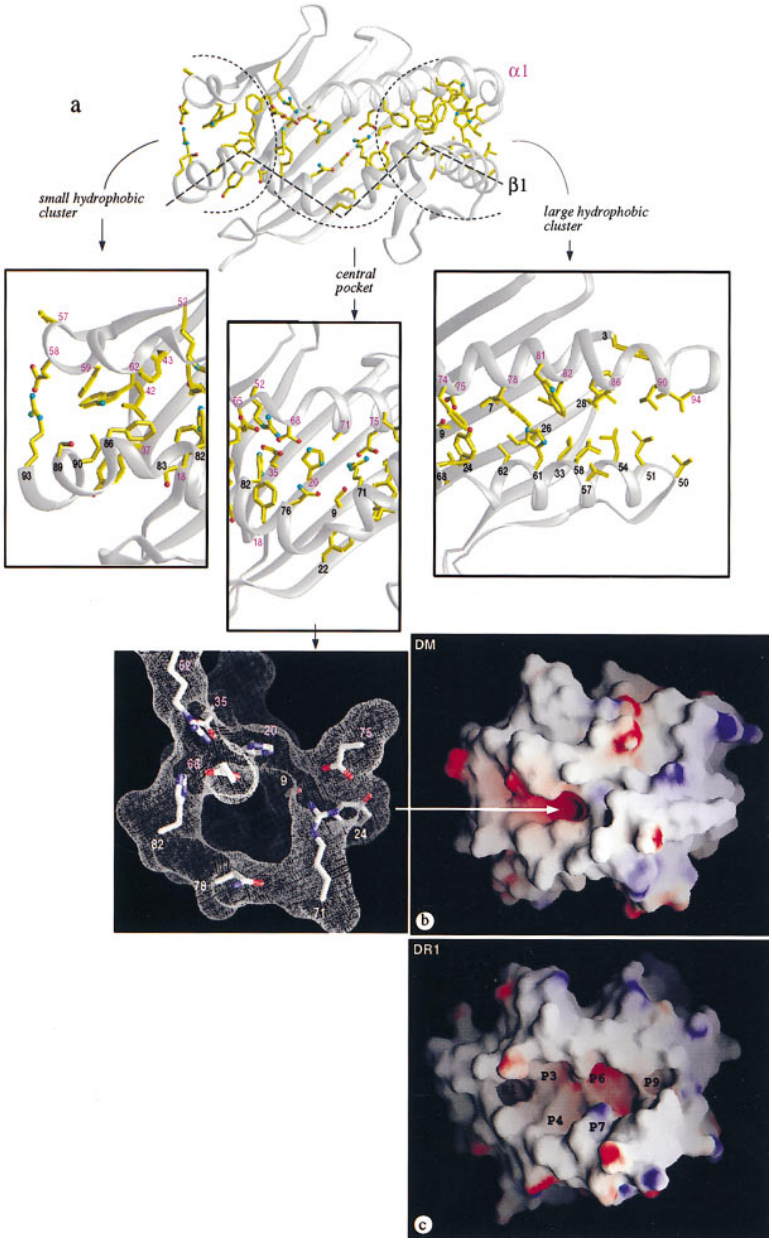

Figure 2. Helical Packing in HLA-DM

(A) Three segments of the helical region pack differently. At each end of the domains the helices contact each other with nonpolar residues; in the middle is a deep pocket of polar residues. At the bottom of the cavity, both Glu α35 and Asp α68 are within hydrogen bonding distance of His α20. The hydroxyl group of Ser β9 could be rotated to hydrogen bond directly to His α20 but in the structure is hydrogen bonded through a water molecule to His α20.

(B) Surface representation of HLA-DM (same viewpoint as in [A]). Arrow shows central deep pocket, also shown in detailed dot surface.

(C) Surface representation of HLA-DR1 (with bound peptide omitted). Peptide side chain binding pockets between the α helices are labeled.

a catalytic site. Whether it is a vestige of such a site or whether it can bind some pendant ligand such as a modified peptide terminus is unknown.

Details of the HLA-DM Structure Are Conserved in Other Species

Most of the residues that fix the α helices of the α1 and β1 domains tightly together and much of the pocket are conserved among human HLA-DM and homologous murine, rabbit, and bovine sequences, while residues that project away from this interface vary and display limited polymorphism within the mouse H2-M alleles. This contrasts with the distribution of conserved and polymorphic positions in the classical class II MHC molecules, where amino acid variations inside the groove permit each MHC allelic product to bind different peptides. The conservation of sequence suggests that all DM homologs will have bulky hydrophobic residues preventing peptide binding at either ends and a pocket, possibly vestigial, at its center.

Besides binding to class II MHC molecules, DM binds intracellularly to HLA-DO, a class II–like heterodimer (Liljedahl et al., 1996; Douek and Altmann, 1997) that evidence indicates is a pH-dependent, negative regulator of DM activity (Denzin et al., 1997; van Ham et al., 1997; Liljedahl et al., 1998). The α and β chains of the inhibitor DO have approximately 60% sequence identity to DR1 (Tonnelle et al., 1985; Trowsdale and Kelly, 1985). One simple model for inhibition by DO would be for both DR and DO to compete for the same site on DM.

A Crystal Packing Dimer of DM

DM crystals contain 2-fold symmetric dimers of αβ DM heterodimers that are probably crystal packing artifacts

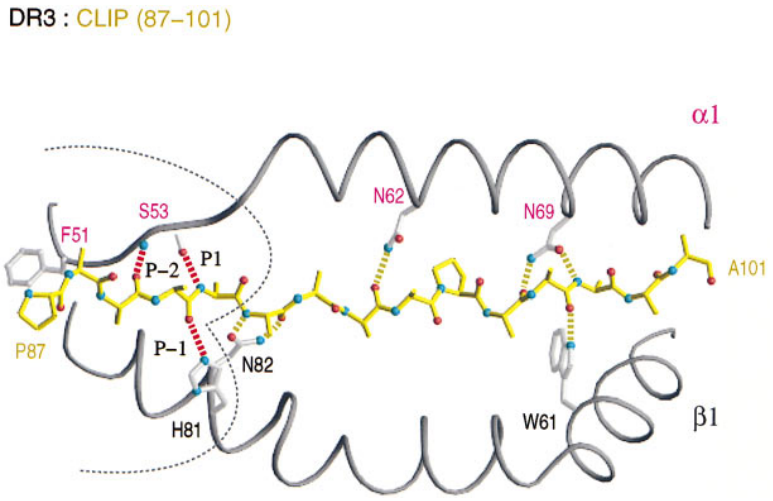

Figure 3. Conserved Hydrogen Bonds between CLIP and HLA-DR3

Dashed lines show hydrogen bonds between peptide backbone atoms found on all bound peptides and residues conserved in all class II molecules, shown for HLA-DR3/CLIP (Ghosh et al., 1995). Hydrogen bonds made by peptide positions 1, −1, and −2 (numbered as in Stern et al., 1994) are labeled P1, P-1, and P-2.

(with a geometry unlike either DR or I-A dimers described previously (Brown et al., 1993; Jardetzky et al., 1994; Stern et al., 1994; Ghosh et al., 1995; Fremont et al., 1996). They are unlikely to be models for either the interaction of DM with DO or with DR (DP and DQ), because the dimers are held together at the membrane distal domains only by contacts involving the oligosaccharides of each α chain (Asn 15, Figure 1A), and DO lacks an equivalent oligosaccharide to form a similar interaction; furthermore, DM from cells with N-glycosylation blocked by tunicamycin is known to catalyze peptide exchange (Guerra et al., 1998).

Discussion

The crystal structure of CLIP (Ii 81–104) bound to HLA-DR3 showed that this intermediate in the maturation of DR had the same conformation as DR complexed with tightly binding peptides, leading to the proposal that to remove CLIP and catalyze peptide exchange, DM must preferentially bind to a strained, transition-state conformer of DR that favors dissociation and association of peptides (Ghosh et al., 1995). A physical association of DM with DR after CLIP dissociation has been observed by immune coprecipitation (Denzin et al., 1996; Sanderson et al., 1996; Kropshofer et al., 1997), suggesting that DM binds tightly to an intermediate, "open-empty" conformation of DR, such as would result after CLIP had been exchanged off. Some of the interactions between the DR molecule and the peptide would be weakened or broken in this open state, facilitating the dissociation or association of peptides.

On the basis of kinetic studies of the DM exchange reaction (Vogt et al., 1996; Weber et al., 1996), Jensen and colleagues showed that the rate of DM-induced dissociation was proportional to the intrinsic rate of dissociation of a peptide for all peptide sequences and different class II allelic molecules tested. This conservation across peptides and class II alleles suggested that some of the bonds that are conserved in all peptide/class II MHC interactions like those involving peptide main chain atoms are the ones disrupted in the DM catalyzed transition state (Weber et al., 1996) rather than peptide side chain/MHC interactions, because those involve polymorphic pockets and allele-specific peptide anchor residues that are different in different class II/peptide pairs. The rate enhancement due to DM could result from DM breaking 2 or 3 of the 11 hydrogen bonds between bound peptide main chain atoms and conserved class II residues observed in all class II/peptide complexes (Stern et al., 1994; Dessen et al., 1997; references therein) (Figure 3).

The three peptide-to-MHC hydrogen bonds nearest to the peptide N terminus (labeled P-2, P-1, and P1 in Figure 3) are good prospects for disruption by DM because of their accessibility on class II structures. Two of them are to an extended strand (Ser α53) of DR that might be displaced more easily than the binding site helices, and one (His β81) extends vulnerably out of the binding-site groove. The six other conserved hydrogen bonds are sheltered within the helical walls of the binding site where their disruption would require more complex distortions of the binding site (Figure 3). The structure of DM reveals one lateral surface that contains two partially exposed tryptophans (α62 and β120; $>50 Å^2$) and is a candidate for binding DR (arrows in Figure 1A). (Fewer aromatic dye molecules bind DM/DR than to either molecule separately, suggesting that nonpolar surfaces form the interface [Ullrich et al., 1997].)

This DM surface (front surface in Figure 1A, left in Figure 3) is homologous to the surface of DR implicated in DM binding by mutation studies (Mellins et al., 1994; Guerra et al., 1998) consistent with the possible formation of a symmetric DM/DR dimer. The addition of an oligosaccharide at DR α94 (Figure 1A) disrupts DM exchange activity both in vivo and in vitro (Mellins et al., 1994; Sloan et al., 1995; Guerra et al., 1998) as well as the physical interaction of DM/DR (Denzin et al., 1996; Sanderson et al., 1996; Kropshofer et al., 1997), without disrupting the overall structure of DR as judged by recognition by T cells (Guerra et al., 1998). The same lateral surface is weakly implicated by the interference with DM activity by molecules thought to bind to near that surface: Cer-CLIP, an antibody to the N terminus of CLIP (Denzin and Cresswell, 1995), and the superantigen TSST-1 (Kim et al., 1994).

A testable although speculative model for DM catalytic activity would be for a nonpolar residue like Trp α62 on this lateral face of DM to contact Phe α51 of DR, which projects off the surface of DR at the N-terminal end of the extended strand characteristic of the "left" end of the peptide-binding sites of class II molecules, and to move neighboring Ser α53, breaking two conserved peptide-to-MHC hydrogen bonds (P-2 and P-1 in Figure 3). In such a hypothetical interaction, a polar residue like Asp α61 (Glu in mouse) of DM might contact His β81 of DR, distorting a third conserved peptide-to-MHC hydrogen bond (P1 in Figure 3). Such a DM/DR interaction could lower the free energy barrier to peptide dissociation and have the properties of an open transition state conformer of DR favoring faster peptide association as well. Mutations at DR His β81 or in the putative DM/DR interface or the structure of DM/DR complex could test this model or, in the latter case, may suggest another mode of destabilizing the peptide/DR interface.

Experimental Procedures

Purification and Crystallization

DM, expressed and isolated as described previously (Sloan et al., 1995), was further purified by gel filtration (Superdex 200) and ion-exchange chromatography (Mono-Q Sepharose, Pharmacia). Two crystal forms were grown at 4°C by combining 2 μl of protein solution (20 mg ml^{-1} in 10 mM TRIS [pH 8.0]) with 2 μl of precipitant (1 M Li_2SO_4, 0.5 M $(NH_4)_2SO_4$, and 0.1 M sodium citrate [pH 5.4]). The structure was determined from an orthorhombic crystal, $C222_1$, a = 95.4 Å, b = 109.9 Å, c = 105.1 Å, with one molecule per asymmetric unit. Diffraction data were collected from a single flash-cooled crystal (100 K), using a Mar Research image plate detector and an Elliot GX-13 rotating anode source with mirror optics. Data were integrated and scaled (Table 1) with DENZO and SCALEPACK (HKL Research). Most of the subsequent processing used the CCP4 programs (Collaborative Computational Project, 1994). An overall isotropic B factor was 38 $Å^2$, with no evidence for anisotropy as estimated from a Wilson plot.

Molecular Replacement and Refinement

DM was located using a model of the HLA-DR1 heterodimer (Stern et al., 1994) in rotation and translation searches with AMoRe (Collaborative Computational Project, 1994). All residues of DR1 were used without alternation, with the HA peptide excluded from the search model. The best rotation and translation solution had a correlation coefficient of 20% and R factor of 53% for all data 12–3.5 Å (next-highest peak, correlation coefficient 13% and R factor 56%). The solution was verified using Patterson correlation refinement in X-PLOR (Brünger and Krukowski, 1990). Initial electron density maps were calculated after the individual domain, rigid body refinement of a search model in which all side chains were truncated to alanines. Extensive manual revision of the model was performed with O (Jones et al., 1991), and refinement and map calculation were done in X-PLOR (Brünger, 1992). At all stages, data from 34.0 to 2.5 Å, with $|F_{obs}| > 0$, were included, with 10% of omitted reflections for R_{free} calculation. The minimization included a bulk-solvent correction coupled with simulated annealing and individual B factor refinement. After four rounds of rebuilding and refinement (R_{free} = 34.3%; R_{cryst} = 25%; 34.0–2.5 Å), the $3F_o$-$2F_o$ and F_o-F_o omit maps, in which helical regions or individual domains of DM were omitted, revealed clear, bias-free electron density for the omitted regions. Continuous electron density was also seen for two N-acetylglucosamine and one mannose moiety at one of the N-linked carbohydrate sites, Asn α15. No saccharides could be modeled at the other expected glycosylation sites, α165Asn and β92Asn. Four subsequent cycles of minimization and rebuilding allowed identification of water molecules from electron density > 2σ in $2F_o$-F_o maps. The final model contained residues α13-199 and β3-192, 198 water molecules, and three monosaccharides (Table 1). All non-glycine ϕ and ψ angles lie in the allowed regions of the Ramachandran plot, with 90% in the most favorable regions. Side chains of residues β139-145 exhibited weak electron density and B factors around 70 $Å^2$. Residues α1-12, β1-2, and those from the C termini, including the epitope-tag peptides, were not detected in the electron density maps, probably because of disordering.

Table 1. Statistics for Data Collection and Refinement

Data collection		
Resolution (Å)	34–2.5	2.59–2.5
Unique reflections	18,729	1,837
Completeness (%)	96.2	96.3
Average I/σ(I)	27.7	6.9
Redundancy	7.3	7.0
Rsym (%)	5.5	21.3
Refinement Results (34–2.5 Å)		
Reflections (free)	16,887(1,842)	
R_{cryst} (R_{free}) (%)	19.4(25.6)	
Non-hydrogen protein atoms	3,002	
Water molecules	198	
Carbohydrates	3(Asn α15)	
R.m.s. deviations		
Bonds (Å)	0.008	
Angles (°)	1.35	
B factors ($Å^2$)	3.5	
Ramachandran Plot Statistics (%)		
Residues in most favored regions	89.8	
Residues in additional allowed regions	9.6	
Residues in generously allowed regions	0.6	
Residues in disallowed regions	0.0	

I/σ(I) is the mean reflection intensity/estimated error. $R_{sym} = (\Sigma|I(i) - <I>|)/\Sigma I(i)$, where I(i) is the intensity of an individual reflection and <I> is the average intensity of that reflection. $R_{cryst} = \Sigma||F_o|-|F_c||/\Sigma|F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes. R_{free} is equivalent to R_{cryst}, but calculated for a randomly chosen 10% of reflections that were omitted from the refinement process.

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