Structural Basis of Sugar Recognition of Misfolded Glycoproteins by OS-9 MRH Domain in ER-Associated Degradation

Misfolded glycoproteins are translocated from ER into the cytosol for ubiquitin proteasome-mediated degradation. A mannose-6-phosphate receptor homology domain of OS-9 (OS-9^{MRH}) is involved in glycoprotein ER-associated degradation (ERAD) through binding to the high-mannose-type glycan. Trimming of outermost α1,2-linked mannose on the C-arm and subsequent binding of processed α1,6-linked mannosyl residues by OS-9^{MRH} are critical steps in guiding misfolded glycoproteins to enter ERAD. One of the key questions in the glycoprotein ERAD system is how OS-9 can specifically recognize the processed high-mannose-type glycans. We performed a crystallographic study of OS-9^{MRH} in combination with NMR and biochemical analyses, obtaining structural insights into this question.

In the endoplasmic reticulum (ER), newly synthesized proteins are subjected to quality control to acquire the correct protein folding. The correctly folded proteins are transported through the ER via the Golgi apparatus to their final destination. On the other hand, potentially toxic misfolded proteins must be destroyed by ER-associated degradation (ERAD) systems. In these systems, *N*-linked glycan plays an important role as a destination signal. For example, ER mannosidases (Man I and EDEMs) regulate ERAD by trimming the mannose residues of the *N*-glycan. Recently, mannose-6-phosphate receptor homology (MRH) domain has been commonly identified in ER lectins OS-9 and XTP3-B, and these lectins were found to be involved in ERAD. The sugar-binding mode of the MRH domain has attracted much attention, and it has been shown that OS-9 and its yeast ortholog Yos9p recognize the terminal α1,2-linked mannose-trimmed C-arm of high-mannose glycan [1-3] [Fig. 1(a)]. However, many questions remain unsolved about the structures and molecular mechanisms. Why is it necessary to process outermost α1,2-linked mannose on this arm for binding? How do MRH domains specifically interact with α1,6-linked mannosyl residues on the C-arm?

We expressed a recombinant human OS-9^{MRH} protein as an inclusion body in *E. coli* and purified it by an oxidative refolding method. The crystallization of OS-9^{MRH} was carried out in the presence of α3,α6-Man$_5$ ligand [Fig. 1(a)], which is a minimal mannosyl oligosaccharide structure of the glycoprotein ERAD substrates processed by ER and/or Golgi α1,2-mannosidases.

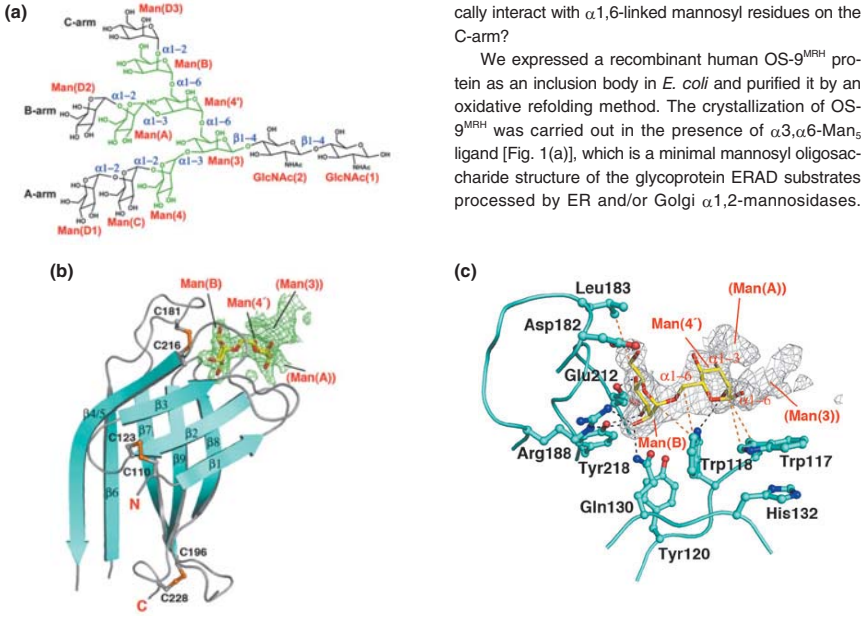

Figure 1
(a) Individual carbohydrate residues of Man$_5$GlcNAc$_2$-Asn are labeled. α3,α6-Man$_5$ used in the crystallization is shown in green. (b) Overall structure of human OS-9^{MRH}. (c) Close-up view of α3,α6-Man$_5$ binding site of OS-9^{MRH}. F_o–F_c omit electron density map of Man(B)-Man(4′) in the α3,α6-Man$_5$-bound complex is shown contoured at 2.2 σ.

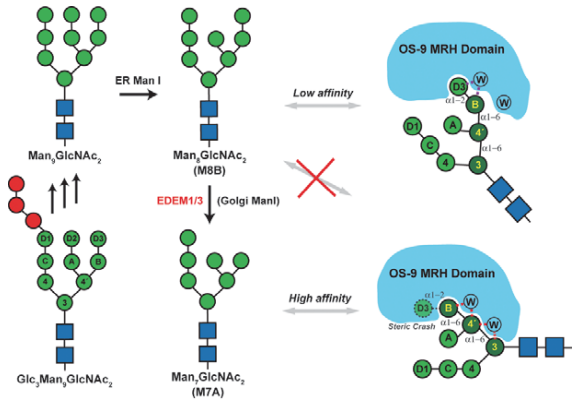

Figure 2
Model for OS-9 recognition of oligosaccharides on ERAD substrates.

Crystals of the OS-9^{MRH}/α3,α6-Man$_5$ complex were obtained in hexagonal lattice $P6_122$ by the hanging drop vapor diffusion method. The crystal structure was solved by single-wavelength anomalous dispersion (SAD) using a crystal of SeCys-substituted protein at 2.85 Å resolution. The SAD data set was collected at the BL-NE-3A. The auxotrophic *E. coli* host cell BL21(DE3) *selB::kan* Cys51E [4] was used for the SeCys labeling experiments. The native data set (2.1 Å resolution) was collected at the NSRRC 13B1 beamline. The refined model of the OS-9^{MRH}/α3,α6-Man$_5$ has an *R*-factor of 22.8% and R_{free} is 27.7% for data between 50.0 and 2.1 Å resolution.

The human OS-9^{MRH} has a flattened β-barrel fold and the overall structure is essentially identical to those of mannose-6-phosphate receptor (MPR) domains [Fig. 1(b)]. Structural conservation of the disulfide bridges between OS-9 and MPRs is observed in the posterior two disulfide bonds (Cys181-Cys216 and Cys196-Cys228 in OS-9), whereas the anterior disulfide bond of OS-9 (Cys110-Cys123) is characteristic of the MRH domain. The α3,α6-Man$_5$-binding site of OS-9^{MRH} is similar to the mannose-6-phosphate binding site of MPRs but additionally includes characteristic continuous double tryptophan residues (Trp117/Trp118, WW motif) for the α6-oligomannose recognition [Fig. 1(c)]. In the Man$_5$-bound OS-9^{MRH} crystal structure, two mannose residues (Manα1,6Man) were visible in the electron density, while the following α1,6-linked Man(3) was disordered due to the crystal packing. We hypothesized that Man(3) is indeed recognized by Trp117 in solution, and hence we performed solution NMR analyses to investigate this interaction. As expected, the NMR analyses revealed that Man(3) interacts with Trp117 and the WW motif is involved in the interaction with Manα1,6Manα1,6Man. Furthermore, site-directed mutagenesis studies showed that OS-9 binds glycoprotein ERAD substrate through the WW motif on the MRH domain.

In summary, our crystallographic and NMR spectroscopic analyses in conjunction with biochemical mutagenesis experiments demonstrate that OS-9 recognizes α1,6-linked trisaccharide Man(B)-Man(4′)-Man(3) residues through the WW motif (Fig. 2, *high affinity*) [5]. Our results provide structural insights into the mechanism of a specific oligosaccharide recognition of high-mannose-type glycans. Trimming of Man(D3) residue is indispensable for this interaction because the presence of Man(D3) leads to steric clashes. Untrimmed glycans may also interact with OS-9 (Fig. 2, *low affinity*), but through fewer contacts—only the disaccharide Man(D3)-Man(B) part could potentially interact with the canonical residues and Trp118.

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BEAMLINE

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