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Structural basis for disparate oligosaccharide-binding specificities in the homologous intracellular lectins ERGIC-53 and VIP36

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1 Introduction

Asparagine-linked oligosaccharides play indispensable roles in the determination of glycoprotein fates in cells through interactions with a variety of intracellular lectins. These lectins specifically recognize partially trimmed processing intermediates of high-mannose-type oligosaccharides displayed on the targeting polypeptide chain and thereby regulate protein folding, degradation, and transport [1]. After correct folding and assembly in the endoplasmic reticulum (ER), the *N*-linked glycoproteins are transported to the Golgi complex by intracellular vesicular transport. Loading of the cargo glycoproteins into the transport vesicles is governed by intracellular lectins, including ERGIC-53 and VIP36. These lectins act as cargo receptors for trafficking certain *N*-linked glycoproteins in the secretory pathway. They share significant structural similarities in their carbohydrate recognition domains (CRDs) but exhibit disparate oligosaccharide-binding specificities and affinities [1]. Namely, VIP36 specifically recognize α1,2-linked D1 mannosyl arm without terminal glucosylation, while ERGIC-53 shows a broader specificity to the high-mannose-type oligosaccharides, irrespective of the presence or absence of the non-reducing terminal glucose residue at the D1 arm. To date, however, the way in which ERGIC-53 shows a broad specificity toward monoglucosylated high-mannose-type oligosaccharides remains largely elusive.

2 Experiments

We purified and crystallized ERGIC-53–CRD as a binary complex with its binding partner MCFD2, a 16-kDa protein possessing two EF-hand Ca^{2+}-binding motifs. To determine the oligosaccharide-bound ternary complex, the crystals of the ERGIC-53–CRD/MCFD2 binary complex were soaked into a reservoir solution containing excess amount of the targeting ligand, α1,2-linked mannotriose (termed α2-Man_3) corresponding to D1 mannosyl arm of high-mannose-type oligosaccharide. The crystal structure was solved by the molecular replacement method with the previously reported binary ERGIC-53–CRD/MCFD2 complex (PDB code: 3A4U) as a search model. The final model of the ternary complex refined to a resolution of 2.75 Å has an R_{work} of 20.2% and R_{free} of 28.7%. We also determined an α2-Man_2-bound complex at 2.60 Å resolution with an R_{work} of 22.0% and R_{free} of 27.3%. The diffraction data sets were collected at Photon Factory BL5A, AR-NE3A, AR-NW12A beamlines.

3 Results and Discussion

Intriguingly, our crystallographic data demonstrated that ERGIC-53 can interact with the D1 trimannosyl arm in two alternative modes, one of which is similar but distinct from that previously observed for VIP36 (Fig. 1) [2]. ERGIC-53 has a shallower oligosaccharide-binding pocket than VIP36 because of the single amino acid substitution, aspartate-to-glycine (Fig. 2). This enables ERGIC-53 to accommodate the non-reducing terminal glucose of the D1 arm in its CRD (Fig. 1, *Mode 2*, and Fig. 2). In the other interaction mode, the 3-OH group of the terminal mannose was situated outward with respect to the sugar binding pocket (Fig. 1, *Mode 1*), also enabling the Glcα1-3 linkage formation without steric hindrance. Our findings thus provide a structural basis for the broad sugar-binding specificity of the ERGIC-53.

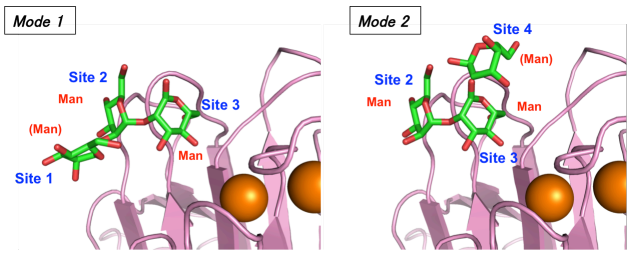

Fig. 1. Two alternative binding modes in ERGIC-53–CRD/oligosaccharide interaction

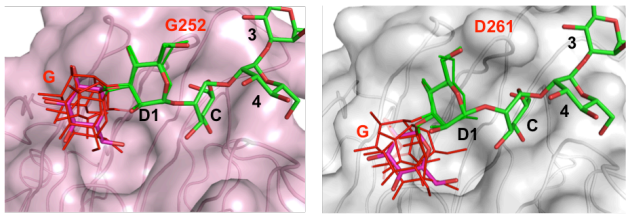

Fig. 2. Models of the homologous intracellular lectins with mono-glucosylated high-mannose-type glycans

References

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