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X-ray Structure of a Protease-resistant Mutant Form of Human Galectin-8 with Two Carbohydrate Recognition Domains

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1 Introduction

Galectin-8 is a tandem-repeat-type β-galactoside-specific animal lectin having N- and C-terminal carbohydrate recognition domains (N-CRD and C-CRD, respectively) with a difference in carbohydrate-binding specificity, involved in cell-matrix interaction, malignant transformation, and cell adhesion. N-CRD exhibits strong affinity for α2-3 sialylated oligosaccharides, giving a feature unique to galectin-8. C-CRD usually exhibits relatively lower affinity for oligosaccharides, but has higher affinity for *N*-glycan-type branched oligosaccharides than does N-CRD. There have been many structural studies on galectins with a single CRD, but no X-ray structure of a galectin containing both CRDs has been reported. We determined X-ray structure of a protease-resistant mutant form of human galectin-8 (G8Null) having both CRDs, in complex with α2-3 sialyllactose (SiaLac) in N-CRD and lactose (Lac) in C-CRD.

2 Experiment

The recombinant G8Null was expressed in *E. coli* BL21 (DE3) and purified by affinity chromatography using a lactose–agarose column. Crystals of G8Null were grown in droplets by mixing 2 μl each of a protein solution (4.3 – 5.9 mg/ml in 10 mM Tris-HCl, pH 7.5, 150 mM NaCl) and a reservoir solution (2 – 3 % (v/v) 1,4-dioxane, 9 – 10 % (w/v) PEG 20000 and 0.1 M bicine pH 9.0) against 450 μl of the reservoir solution, by the hanging drop method at 293 K. Data collection for G8Null was done on PF-AR NW12A and BL-5A (KEK, Japan) using an ADSC Quantum 210r or 315 CCD detector at a wavelength of 1.0 Å. The initial phases were determined by molecular replacement, and the structure was refined using 2.98 Å resolution data.

3 Results and Discussion

In G8Null structure, Lac molecules, which are introduced during the protein purification, have bound to both N- and C-CRD. After many attempts at soaking of SiaLac to G8Null crystals, a G8Null complex including SiaLac was successfully obtained, in which a SiaLac and a Lac bind to N- and C-CRD, respectively (G8Null-SiaLac-Lac). The structure of G8Null-SiaLac-Lac is shown in Fig. 1A. G8Null consists of two domains (N- and C-CRD), which are connected by two amino acid residues (His-Met), instead of a linker region of 28 residues. The galectin CRD adopts a β-sandwich structure formed by two anti-parallel β-sheets of six (S1-S6) and five (F1-F5) β-strands, with a short α-helix located between F5 and S2. Oligosaccharides (SiaLac and Lac) bind to the concave surface formed by S3, S4, S5, and S6, in a similar manner to those found in other galectin-oligosaccharide complexes. The N-CRD of G8Null has two additional β-strands, F0 and S0. F0 of Ile11 – Asn14 is part of the (F1-F5) β-sheet of N-CRD. Interestingly, S0 of Ser4 – Asn6 interacts with S1 of C-CRD, with three hydrogen bonds between main chains (Ser4 – Asn192 and Asn6 – Arg190) and additional hydrogen bonds with side chain groups of Asn residues (Leu3 – Asn192 and Asn7 – Ala188) (Fig. 1B). This S0 – S1 inter-domain interaction should be related to the spatial arrangement of the two CRDs; directions of carbohydrate-binding sites of N- and C-CRD make an almost right angle.

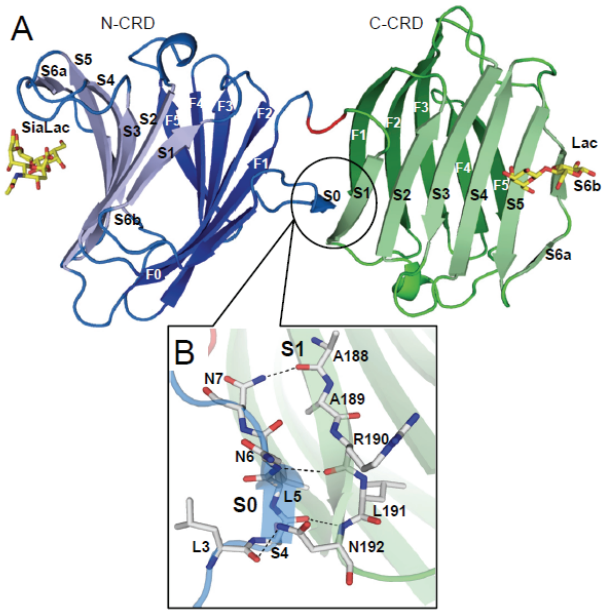

Fig. 1: Overall structure of G8Null. (A) Structure of G8Null with the bound SiaLac and Lac is shown. N-CRD, C-CRD, and the linker (His-Met) are indicated by blue, green, and red, respectively, and (S1-S6) β-sheets are in light colors. (B) Interactions between S0 and S1 in G8Null are shown with hydrogen bonds.

References

[1] H. Yoshida *et al.*, *FEBS J.* **279**, 3937-3951 (2012).

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