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X-ray structure of galectin-9 C-terminal domain in complex with sialyllactose

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Introduction

The galectins are a family of β-galactoside-specific animal lectins which contain conserved elements for carbohydrate recognition. Currently, there are 14 members of the mammalian galectin family, classified into three subtypes on the basis of structure. The prototype (galectin-1, -2, -5, -7, -10, -13, -14, and -15) has a single carbohydrate recognition domain (CRD), that usually forms a non-covalent homodimer. The chimera-type (galectin-3) has a single CRD with an extended N-terminal nonlectin domain. The tandem-repeat-type galectins (galectin-4, -6, -8, -9, and -12) have two CRDs, one each in the at N- and C-terminal regions, joined by a linker peptide. Tandem-repeat type galectin-9 is involved in chemoattraction, apoptosis, and the regulation of cell differentiation, and has anti-allergic effects. Interestingly, human galection-9 C-terminal CRD (hG9C), as well as galectin-8 N-terminal CRD (hG8N), has significant affinity for the α2-3 sialylated oligosaccharides, but any other galectins do not. To elucidate the recognition mechanism of α2-3 sialylated oligosaccharides by hG9C, X-ray structure of hG9C in the complex with α2-3 sialyllactose (SiaLac) was determined [1].

Materials and methods

The expression, purification and crystallization of hG9C have been reported [1]. Data were collected at KEK PF-AR NW12A and BL-5A, using an ADSC Quantum 210r or 315 CCD detector with a wavelength of 1.0 Å. All data were processed using the HKL2000 system. Crystals of complexes with oligosaccharides were obtained by a soaking method. Aqueous solution of SiaLac (400 mM) was added to droplets of the crystals (0.2 – 0.4 μl), incubated for 2 days. Molecular replacement was applied with the program MOLREP. The initial phase was determined using human galectin-3 CRD (PDB code: 1KJL) which has 42 % sequence identity with hG9C.

Result and discussion

The carbohydrate-binding site with the bound SiaLac is shown in Fig. 1A. The α2-3 glucoside bond between Sia^{-1} and Gal^{+1} is bent extensively, placing Sia^{-1} beyond the concave surface of the carbohydrate-binding site. Interestingly, Arg221 adopts two conformer (1 and 2), and forms hydrogen bonds with the carboxyl group and the glucoside oxygen atom of Sia^{-1} in conformer-2, efficiently recognizing Sia^{-1} residue. Asp241 forms strong hydrogen bonds with Arg221 in conformer-1 (Fig. 1B), partially preventing Arg221 from changing its conformation, and this may lead to the two conformers of Arg221 in hG9C/SiaLac. The hG8N has great affinity for SiaLac, and the X-ray structure of hG8N in a complex with lactose is available for the structural comparison of hG9C with hG8N. Instead of Arg221 and Asp241, hG8N has Arg52 and Lys78, which are incapable of attractive interactions. These results suggest that Arg221 of hG9C is responsible to recognize sialylated oligosaccharides, and that the replacement Asp241 by another amino acid may give a strong affinity for sialylated oligosaccharides.

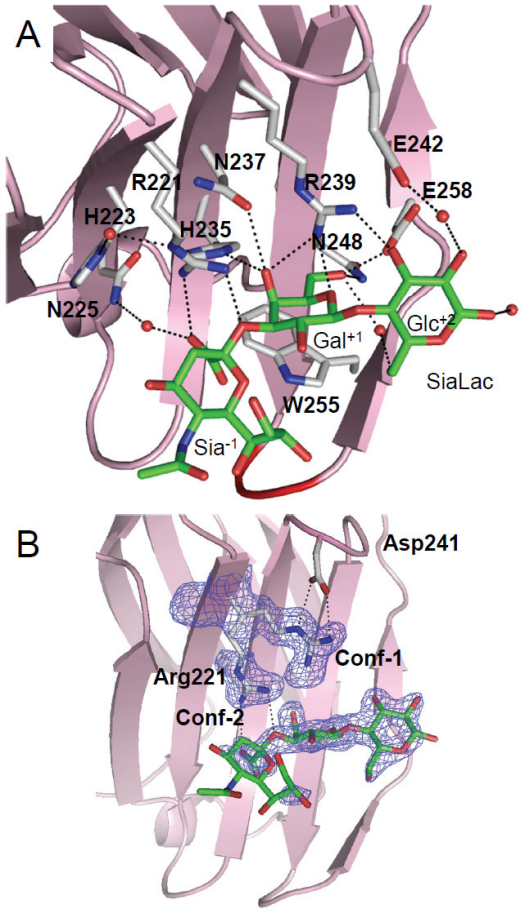

Figure 1. (A) Structure of carbohydrate-binding sites of hG9C with SiaLac. (B) Two conformers of Arg221, interacting with Asp241 (conformer-1) and SiaLac (conformer-2).

Reference

[1] H. Yoshida et al., *J. Biol. Chem.*, **285**, 36969-36976. (2010).

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