Crystal structure of a *Xenopus laevis* skin prototype galectin

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1 Introduction

Galectins, a β-galactoside-specific soluble lectin family, are widely distributed in metazoans and fungi. The galectin family members show a variety of expression patterns, ligand specificities and biological functions. Mammalian galectin-1, one of the most studied galectins, is expressed in many tissues and cells, and is involved in many functions such as induction of cell death, cell adhesion and differentiation. *Xenopus laevis* (African clawed frog) has two types of galectins that are similar to mammalian galectin-1 in amino acid sequence. One type, comprising xgalectin-Ia and -Ib, is regarded as being equivalent to galectin-1, and the other type, comprising xgalectin-Va and -Vb, is expected to be a unique galectin subgroup. The latter is considerably abundant in frog skin; however, its biological function remains unclear. We determined and reported the crystal structures of xgalectin-Ib and -Va [1]. The structures showed that both galectins formed a mammalian galectin-1-like homodimer, and furthermore, xgalectin-Va formed a homotetramer. This tetramer structure has not been reported for other galectins. To elucidate intermolecular interactions in a tetramer, more precisely, the structure of xgalectin-Va at a higher resolution has been determined.

2 Experiment

The recombinant xgalectin-Va was expressed in *E. coli* BL21 cells, and purified by affinity chromatography (a glutathione-Sepharose 4B column and a lactose–agarose column). Large single crystals of xgalectin-Va were obtained with a protein solution (5.0 mg/ml in 20 mM Tris–HCl (pH 7.2), 50 mM NaCl, 1–2 mM DTT) and a reservoir solution (2.4 M sodium malonate (pH 5.0)) by the sitting drop method at 293 K. Data collection was done on PF-AR NW12A (KEK, Japan) using an ADSC Quantum CCD detector at a wavelength of 1.0 Å. The structure was solved by an isomorphous replacement method using the previously reported structure at 1.60 Å resolution (3WUC), and was refined to R-factor of 0.196 (Rfree = 0.216), using 1.36 Å resolution data.

3 Results and Discussion

Structure of xgalectin-Va comprised a β-sandwich composed of six-stranded (S1–S6) and five-stranded (F1–F5) β-sheets, which is typical of the galectins so far reported. A lactose molecule, which was introduced during the protein purification, was found in a carbohydrate-binding site. Two molecules of xgalectin-Va formed a homodimer, in which two intermolecular β-sheets were formed by the F1 and S1 strands, as found in galectin-1 (Fig. 1A). The tetramer was formed through the symmetric association of two dimers (Mol-A/B and Mol-C/D) (Fig. 1B). The dimer-dimer interface mainly involved the F2 and F3 strands, and the S6–F3 and F4–F5 loops. Four carbohydrate-binding sites are located on the surface of a tetramer. Thus, the tetramer formation with four carbohydrate-binding sites may be related to the function of xgalectin-Va. In the newly determined structure at 1.36 Å resolution, some amino acid residues in the dimer-dimer interface were found to adopt alternative conformations of the side chain, which may contribute to the intermolecular interactions in a tetramer.

Fig. 1: Crystal structure of xgalectin-Va. (A) Dimer structure formed by Mol-A/B is shown with the bound lactose molecules. (B) Tetramer structure is shown.

Acknowledgement

We thank the PF staff for support of data collection.

References


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