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X-ray structure of a protease-resistant mutant form of human galectin-8

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Introduction

Galectin-8 is one of the tandem-repeat-type galectins having two carbohydrate recognition domains (CRDs) joined by a ~30 amino acid linker peptide. Galectin-8 is expected to be involved in malignant transformation and cell-matrix interaction, and it was also reported to regulate neutrophil adhesion by interacting with target molecules. The tandem-repeat-type galectins are susceptible to proteolysis due to the presence of the linker peptides. A mutant form of human galectin-8 (G8Null) that lacks the entire linker region has a high protease-resistance compared to wild-type galectin-8, and interestingly it can induce neutrophil adhesion in the same manner as wild-type galectin-8. This suggests that the linker peptide is not essential for interaction with target molecules in the regulation of cell adhesion. To elucidate the relationship between the structure and biological activity of galectin-8, the structure of a galectin-8 with both N- and C- terminal CRDs is very important.

Materials and methods

Initial crystallization screening for G8Null was performed using Crystal Screen kits I & II, PEG/Ion Screen, SaltRX Screen, Index Screen (Hampton Research Corp., CA, USA), and Emerald BioSystems Wizard I & II & III (Emerald BioSystems, Inc., WA, USA) by the sitting drop method at 293 K. Small crystals appeared under the condition of mixing each 1 µl of protein solution (4.26 mg/ml in 10 mM Tris-HCl, pH 7.5, 50 mM NaCl) and reservoir solution, 2 % (v/v) 1,4-dioxane, 10 % (w/v) PEG 20000 and 0.1 M bicine pH 9.0 (Crystal Screen II - 48) in two weeks. A crystal with the dimensions of 0.05 × 0.05 × 0.05 mm was grown in a droplet consisting of a mixture of each 2 µl of protein solution and above reservoir solution (Crystal Screen II - 48) after 6 days by the hanging drop method [1].

Data collection was carried out at the KEK PF-AR NW12A using an ADSC Quantum 210 CCD detector at a wavelength of 1.0 Å. A crystal mounted in a loop was soaked in cryoprotectant solution containing 30 % (w/v) glycerol and flash-cooled in a stream of evaporating nitrogen at 100K. All data were processed using HKL2000.

A complete data set at 3.16 Å resolution was successfully collected. The crystal belongs to the tetragonal space group $P4_12_12$ or $P4_32_12$, with cell dimensions of a = 78.93 Å, b = 78.93 Å, c = 132.05 Å. The asymmetric unit is expected to contain a molecule with the crystal volume per unit molecular weight of V_M, 3.1 Å^3 Da^{-1}, corresponding to a solvent content of 60.5%. Using a structure of human galectin-8 N-terminal domain (PDB code, 2YV8), a molecular replacement method was applied by the Molrep program in CCP4 program suite, giving a clear solution of the rotation and translation parameters for two CRDs in the space group of $P4_32_12$. On the electron density map obtained with the resultant phases, the main chain structure of Ser4 - Trp291 could be built, and also many of the side chain groups could be located, using the program Coot in CCP4 program suite. The structure was refined to an R-factor = 0.33, using 3.16 Å resolution data.

Result and discussion

The overall structure of G8Null is shown in Figure 1. Two CRDs are related by pseudo 2-fold symmetry, and carbohydrate binding sites are located at both ends. So far, the crystal structures of a single CRD of tandem-repeat-type galectins (galectin-4, -8, and -9) have been determined, and this is the first report of an X-ray structure of a tandem-repeat-type galectin that includes both CRD.

We are currently refining the crystallization conditions to obtain higher diffracted crystals.

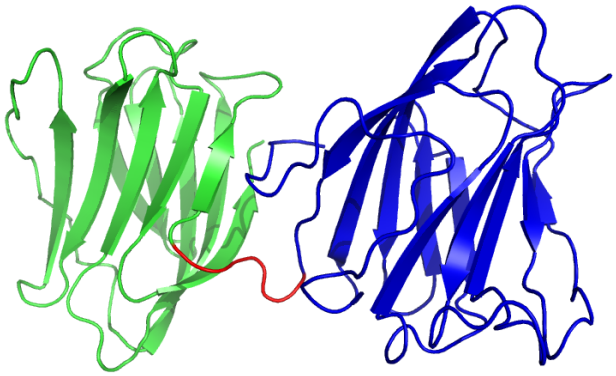

Figure 1. The overall structure of G8Null is illustrated by the program PyMol. The N- and C-terminal CRDs are shown in green and blue, respectively. The linker loop is shown in red.

References

[1] H. Yoshida et al., *Acta Crystallogr. Sect F* 65, 512-514. (2009).

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