X-ray crystallographic analysis of the C-type lectin CEL-I and its complex with carbohydrates

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Introduction

Carbohydrate-binding proteins (lectins) have been known to play important roles in various tissues and body fluids for molecular recognition processes. There are increasing number of animal lectin families, which are categorized according to their amino acid sequence homologies. Among these, C-type lectin family includes various proteins that exert carbohydrate-binding activity depending on the presence of Ca$^{2+}$ ion. We have isolated four Ca$^{2+}$-dependent galactose/N-acetylgalactosamine-specific lectins (CEL-I, II, III, and IV) from the marine invertebrate Cucumaria echinata (Holothuroidea) [1]. Among these, CEL-I and -IV were found to belong to C-type lectin family. CEL-I, which is the smallest lectin in C. echinata, is composed of two identical subunits of 16 kDa with a single interchain disulfide bond, and has very high specificity for N-acetylgalactosamine; the affinity for GalNAc is about 1000 times higher than that for galactose, as judged by the hemagglutination inhibition test. We have already crystallized CEL-I and preliminarily analyzed its X-ray crystal structure by molecular replacement method using human lithostathine as a search model [2]. In order to elucidate the carbohydrate-recognition mechanism, we have tried to analyze crystal structures of CEL-I/carbohydrate complexes.

Results and Discussion

The crystals of CEL-I/GalNAc complex were prepared by the hanging drop vapor diffusion method in 0.1 M Tris-HCl, pH 8.0, 60% MPD at 20°C. Crystals belong to the space group $P2_1$ with unit cell dimensions $a = 39.58$ Å, $b = 52.52$ Å, $c = 136.96$ Å, $\beta = 91.63^\circ$. There are two molecules of CEL-I dimer in the asymmetric unit. The structure of CEL-I/GalNAc complex was solved by the molecular replacement method using native CEL-I as a search model. Recognition of GalNAc by CEL-I is basically done through coordinate bonds between 3- and 4-OH of the sugar and one of two Ca$^{2+}$ ions in the CEL-I protomer as well as hydrogen bonds of these hydroxyl groups with Gln101, Asp103, Glu109, and Asn123. In addition to these bonds, high affinity binding of GalNAc to CEL-I is achieved by formation of two hydrogen bonds between the side chain of Arg115 and carbonyl oxygen of the acetamido group of GalNAc. On the other hand, we have also prepared crystals of CEL-I/lactose complex.

References


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