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

Tomomitsu HATAKEYAMA*[1], Tatsuya UCHIDA[2], Hajime SUGAWARA[3], Masami KUSUNOKI[2]
[1]Department of Applied Chemistry, Faculty of Engineering, Nagasaki University, Nagasaki 852-8521, Japan, [2]Institute for Protein Research, Osaka University, 3-2 Yamada-oka, Suita, Osaka 565-0871, Japan, [3]Laboratory for Communication Mechanism, RIKEN Plant Science Center, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan

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, β= 91.63°. 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. The crystals of CEL-I/lactose complex were prepared by hanging drop and sitting drop vapor diffusion methods in 0.1 M Tris-HCl, pH 8.0, 50-60% MPD at 20°C. Initially, microcrystals were prepared using the protein solution without lactose, and then they were used as seeds to form larger crystals using the protein solution containing 10 mM lactose. Crystals of appropriate sizes were obtained within two weeks. Crystals belong to the space group *C*2 with unit cell dimensions a = 114.91 Å, b = 59.35 Å, c = 112.59 Å, β= 129.45°. There are two molecules of CEL-I dimer in the asymmetric unit. The structure of CEL-I/lactose complex was solved by the molecular replacement method using native CEL-I as a search model. In the complex, lactose molecule was basically recognized through the 3- and 4-OH groups of its galactose moiety, forming coordinate bonds with a Ca^{2+} ion located at the carbohydrate-binding site of CEL-I, as well as four hydrogen bonds with amino acid residues in the vicinity. On the other hand, there is little interaction between glucose moiety of lactose and the carbohydrate-binding site of CEL-I. These results are consistent with the observation that lactose has much less binding affinity with CEL-I, compared with N-acetylgalactosamine.

References

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* thata@net.nagasaki-u.ac.jp