Crystallography

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Crystal structure analysis of new-type lectin from algae

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Introduction

Recognition between proteins and carbohydrates is of prime importance in many biological processes. Legume lectins are well-studied proteins because they are not only easy to purify in large quantities but also exhibit a wide variety of carbohydrate specificities despite strong sequence conservations.

But lectins from algae are quite hard to purify and even its existence was not known for a long time. For example, the review of "Lectin", which was published in 1986, did not describe lectins from algae. Hori *et al*. first succeeded the purification of algae lectin, and now the number of species is over 20. Lectin from algae was also found to have the anti-cancer effect in the medical experiment for the mouse.

Results and Discussion

Thus, we first crystallized one of the algae lectins, ESA-2. The crystals were obtained by hanging-drop vapor diffusion method at 20 °C, but the shape was quite thin plate and the thickness was less than 10 μm. Thus, X-ray from generator is too weak for the diffraction study and the strong beam from synchrotron was expected to be useful.

The strong synchrotron beam using undulator was quite effective for such a thin crystal and the diffraction was observed over 1.5 Å resolution. As the highest resolution of legume lectin is 1.8 Å resolution, the more precise structure determination will be expected. This crystal belonged to the space group $P2_1$ and the lattice parameter was determined to a = 42.20Å, b = 62.43Å, c = 48.53Å and β = 110.34°. Diffraction images were digitalized and merged using the program d*TREK or MOSFLM.

The three dimensional structure of ESA-2 was determined by multiple isomorphous replacement method using diffraction data of 2.0 Å resolution. Crystallographic refinement were performed using REFMAC and the R and R_{free} were converged to 0.179 and 0.223, respectively. The structure is quite unique comparing with the higher plant lectins. The four sequential repeat exists in the amino acid sequence. The four repeated structure is quite well superimposed, and this fact agrees with that this protein molecule has four high mannose-specific binding sites. In the high plant lectin, the binding site is formed by subunit structure, but in the case of ESA-2, the binding site is formed by domain structure. By comparing with the high mannose-specific lectin family, the binding site is obviously predictable by comparing with the new-lectin family.

Comparing with the higher plant lectin, *Galanthus nivias* allugutin (GNA) is most similar to ESA-2, although GNA binds with sugar by forming dimmer. The sequence of GNA at Gln26, Asn30, Val32, Tyr34 on the β-strand are similar with Tyr3, Val5, Asn7, Gln8 on the β-strand by seeing inversely. But in order to clarify the specific characters of ESA-2, such as only high mannose speficity, non-metal requirement are still unknown.

Thus, we are now searching for the condition of obtaining larger crystal, and trying to make the complex crystal with the carbohydrate in order to clarify the unique high mannose-specific recognition mechanism of this lectin family.

References

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