Verification of Okuyama model for collagen based on the structure analyses of model peptides, (X-Y-Gly)n

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Among the structural models proposed for native collagen, the Rich and Crick model (10/3-helical model) and the Okuyama model (7/2-helical model) have been generally accepted. Although the latter was derived from the structure analysis of the model peptide, $(Pro\text{-}Pro\text{-}Gly)_{10}$ single crystal, this could explain the fiber diffraction pattern of native collagen. Recently, structural studies on other model peptides, such as $(Pro\text{-}Hyp\text{-}Gly)_{10}$, $(Pro\text{-}Hyp\text{-}Gly)_4$-(Pro-Hyp-Ala)-$(Pro\text{-}Hyp\text{-}Gly)_5$ and so on, have revealed the overall structures of peptides to be in accordance with the Okuyama model. In order to elucidate the details of collagen structure and confirm the Okuyama model, we are currently investigating a series of the crystal structures of collagen model peptides. During the present beamtime, we collected the intensity data of $(Pro\text{-}Pro\text{-}Gly)_9$, $(Pro\text{-}Hyp\text{-}Gly)_9$, and $(Pro\text{-}Pro\text{-}Gly)_4$-(Pro-Arg-Gly)-$(Pro\text{-}Pro\text{-}Gly)_4$ (hereafter referred to as PPG9, POG9, and PRG, respectively).

Crystals of PPG9, POG9, and PRG were grown by hanging drop vapor diffusion method using PEG400 as a precipitant. X-ray diffraction experiments were performed at BL44XU of the SPring-8 synchrotron radiation source. Diffraction data were recorded on Oxford PX210 CCD detector system with a total oscillation range of 180° at 100 K. The oscillation angle and exposure time per flame were 1.0° and 10 sec, respectively. Intensity data were processed using DPS/MOSFLM program. Data collection and processing statistics of PRG are shown in Table 1. Structure analysis is now in progress.

Table 1. Summary of data collection of PRG

Wavelength / Å	0.9
Camera distance / mm	174
Camera Z-trans / mm	40
Cell dimension	
a / Å	21.95
b / Å	22.15
c / Å	29.51
β / °	100.36
Resolution / Å	1.49
No. of unique refs.	4328
R_{merge}	0.138
Completeness / %	92.7

Crystal structure analysis of new-type lectin from algae

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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.

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 was determined to belong 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. In this beam time, over 12 derivative crystals were used for data collection and heavy atom search. But only the K_2PtI_6 derivative was found to give the clear difference Patterson peak on the Harker section, as shown in the figure. Thus, we are now searching another good derivatives for MIR method.

We are now searching the condition of obtaining larger crystal, and trying make the complex crystal with the carbohydrate in order to clarify the unique recognition mechanism of this enzyme.