Application of X-ray powder diffraction to protein crystallography -II

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We have continued to collect X-ray powder diffraction data of protein crystals. These trials is interesting that it will be able to analyze structure using only microcrystal and only single frame data with short-time exposure. This time we have focused to search the condition to determine the protein and inhibitor complex with sorking method into the microcrystal.

We have selected bovine pancreas trypsin, which is crystallized with a inhibitor (benzamidine)in addition to $CaCl_2$ and ammonium sulfate at pH 7.0. After releasing benzamidine from the crystal in a precipitant solution, another inhibitor (leupeptin) is sorked. All microcrystals were made rapidly with its size of 1-2 micormeter, using the same precipitant and pH for making single crystals.

The data collection is executed by using R-AXIS IV^{++} (Imaging plate system) with 400 mm vacuum path with 3mm beam stopper for SAXS experiment, which is a merit of low level background.

Using all micro crystals centrifuged in glass capillary, clear powder rings are detected with 5 degree oscillation for 5 to 60 minutes exposure time. The diffraction is determined up to 3 Å resolution with 1.000 Å wavelength.

Between trypsin with benzamidine and trypsin complex with leupeptin, there is significantly difference in intensity of some peaks within 10 degree of 2 theta angle.

The single crystals diffraction data of trypsin with benzamidine and trypsin complex with leupeptin were also collected using CCD camera ADSC Quantum 4R with 1.000 Å wavelength without SAXS path at 2.4 Å resolution. Its space group is $P2_12_12_1$ and its cell parameters are a=63.54 Å, b=69.08 Å, c=61.82 Å.

The structure refinement using powder diffraction data comparing with a refined single crystal structure is now under going.

In contrast to co-crystallization, the sorking method will be widely useful to compare many inhibitors complex structure with a enzyme. This suggests that the rapid structure determination for drug design will be able to apply using micro crystals.

In addition, we are going to develop a new beam path with 1000 mm long and 30 degree with 2 theta angle to separate overlapped diffraction with protein crystal.

Small Angle X-ray Scattering from a Dual-Component Organogel to Exhibit Charge Transfer Interaction

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Organogelators are a class of molecules that can undergo self-organization in a particular organic solvent to yield a fine febrile structure. We have studied on a series of sugar-appended organogelators.[1-4] In this work,[4] we found that the compounds (I) and (II) forms a dual component gel with the charge-transfer interaction, hereinafter we denote (I) and (II) as p-NO2Glu + p-NH2Glu, respectively. This paper explores how the supramolecular structure in the gel changes in accordance with the composition change.

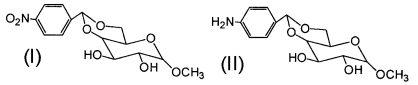

Figure 1. Chemical structures of the charge transfer gels (I) and (II).

Figure 2 compares the SAXS profiles among different compositions for p-NO2Glu + p-NH2Glu in diphenyl ether at room temperature. In the individual gels, sharp peaks due to the crystalline long periods can be observed, at 3.60, 3.77, and 4.62 m^{-1} for the p-NH2Glu gel, and 3.72, 4.33, and 4.79 nm^{-1} for the p-NO2Glu gel, respectively. On the other hand, the stoichiometric dual-component gel (50:50) shows no crystalline peak at all, but two broad scattering oscillations are observed around 0.8 and 1.9 nm^{-1}. This feature is characteristic for the particle scattering. In the p-NH2Glu rich gels; 20:80 and 40:60, the 3.77, and 4.62 m^{-1} peaks remain, indicating that the pure p-NH2Glu crystalline still exists the dual component. On the other hand, there is no crystalline-originated sharp peak in the p-NO2Glu rich gels; 80:20 and 60:40. However, careful examination reveals that there are broad peaks observed in the 80:20 gels around 3.5, 4.2, and 5.0 nm^{-1}. These peak-widths are considerately broad and the peak positions are not same as those in the original p-NO2Glu gel, suggesting that another structure or some defect is present.

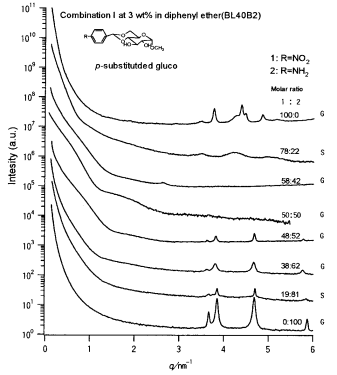

Figure 2. Composition dependence of the dual component gel in diphenyl ether at room temperature.

The scattering feature in Figure 2 is consistent with the fact that p-NO2Glu and p-NH2Glu form 1:1 complex with the charge transfer interaction. Furthermore, SEM and optical microscopy showed that the individual components consist of fibril-like micro crystalline, on the other hand, the 50:50 gel, very fine fibrils.

(1) Shinkai et al., *Chem. Eur. J.* **2001**, *7*, 4328. (2) Gronwald et al., Carbohydrate Res., **2001**, *331*, 307. (3) Sakurai et al., Chem. Letter **2001**, 748. (4) A Frigger et al., *J. Am. Che, Soc.*, in press.