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Colloidal crystallization of highly-concentrated protein molecules in water

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Introduction

Although most of protein crystals have been grown using precipitants, many "soft" proteins have not been crystallized. This is mainly due to the fluctuation of the structure of the above "soft" proteins.

If we can crystallize protein molecules as a colloidal crystal, these "soft" proteins will be crystallized. The spatial separation between proteins in the colloidal crystal will be useful for the adjustment of the regular spatial positions of the "soft" proteins.

In this study we tried to make a colloidal crystal of a protein molecule by centrifugal condensation in ultrapure water and dilute aqueous HCl solutions.

Experimental

Hen egg-white lysozyme (Seikagaku-Kogyo, 6 times recrystallized) was used as a model protein without further purification. 0.6 g lysozyme was dissolved in 15 mL solvents (ultrapure water (> 18.2 MΩcm), 0.001 and 0.01 M HCl aq.) and centrifugal condensation was done. This procedure was repeated three times, and finally 300 μL of ~200 mgmL^{-1} lysozyme solution was obtained.

Diffraction data from the highly-concentrated lysozyme solution were collected using a synchrotron radiation at BL-5A of KEK-PF.

Results and Discussion

Centrifugal condensation of lysozyme up to ~200 mgmL^{-1} was successfully done at 25 °C. Lysozyme which was dissolved in 0.01 M aqueous HCl solution was stable throughout this study. However, in the other cases, needle-like crystals were nucleated and grown (Fig. 1)

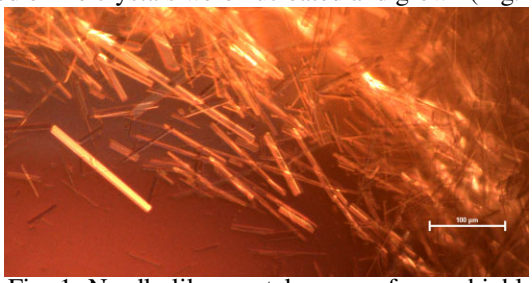

Fig. 1. Needle-like crystals grown from a highly-concentrated lysozyme solution in ultrapure water. Scale bar represents 100 μm.

These crystals were nucleated without the aid of any precipitating agents. The crystals dissolved when the temperature increased up to 30 °C. Thus, the interaction in the crystals is attractive, since the heat of dissolution is positive. Retailleau et al. reported that they prepare lysozyme crystals without precipitant (NaCl) and measure the solubility of the crystals [1], however, they have not shown further information about the precipitant-less protein crystallization technique and the 3D structure of lysozyme in the crystals

One degree oscillation photographs of lysozyme molecules dissolved in 0.01 M HCl are shown in Fig. 2.

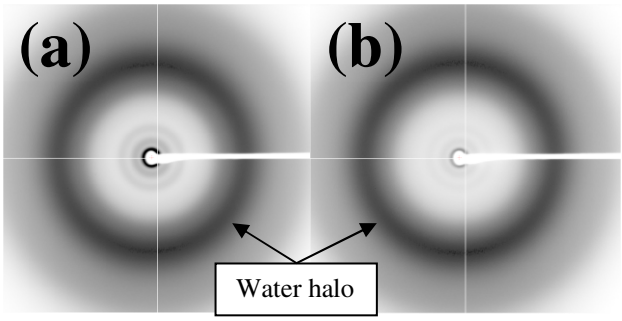

Fig. 2. One degree oscillation photographs of (a) 239 mgmL^{-1} and (b) 80 mgmL^{-1} lysozyme solutions.

Although the intensity of three rings inside a strong water halo increased with lysozyme concentration, radii of them did not change. Thus these rings are not originated from colloidal polycrystal, but are small angle scattering from lysozyme solution.

A one degree oscillation photograph of needle-like lysozyme crystals dissolved in ultrapure water is shown in Fig. 3. Clear diffraction spots indicate that the crystallinity of needle-like crystals is high.

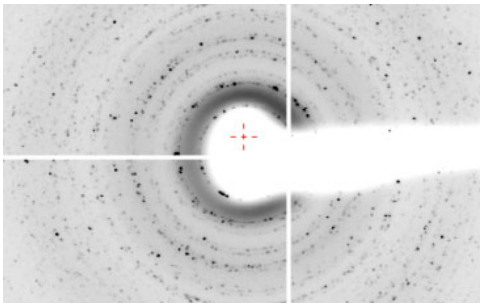

Fig. 3. A one degree oscillation photograph of needle-like crystals shown in Fig. 1. Three lysozyme halos shown here correspond to those in Fig. 2.

Conclusions

Hen lysozyme molecules dissolved in ultrapure water, 0.001 M HCl aqueous solution, and 0.01 M HCl aq. were successfully concentrated by centrifugal condensation. One degree oscillation photograph of highly-concentrated lysozyme solution did not show the evidence of the existence of colloidal crystals, while we successfully obtained lysozyme crystals without using any precipitants.

References

[1] P. Retailleau et al., Biophys. J. 73, 2156 (1997).

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