Protein crystallization induced by poly(ethylene) glycol: A small angle x-ray scattering study

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水溶性高分子が球状タンパク質分子間にどのような相互作用をどのようなメカニズムで誘起するのかを知ることは、タンパク質溶液の制御（安定化や結晶化など）のために重要である。また、通常電荷を持ち、静電的に安定化しているタンパク質溶液の構造を、電荷を持たない水溶性の高分子が不安定化するメカニズムはソフトマターの液体構造という視点からも興味深い。本研究では、モデルタンパク質として直径約8nmのグルコースイソメラーゼを用い、水溶性高分子ポリエチレングリコールが誘起するタンパク質の溶液構造変化を、X線小角散乱法によって測定した。

Proteins in aqueous solutions usually have surface charges which stabilize the solution. By adding salt or changing pH of the solution to decrease the electrostatic stabilization induces the molecular aggregation. On the other hand, it is well known that water-soluble polymers such as polyethylene glycol can destabilize protein solutions. Since these polymers often do not have any charges, it is believed that the primary mechanism of the destabilization is the depletion attraction at so-called protein limit [1], where the size of polymers is much larger than that of proteins. The real situation is, however, far more complicated than the model in theories. For example, the size of typical proteins (a few nanometers) is more or less similar to that of polymers frequently used for protein solutions. Moreover, as mentioned above, proteins have charges, which can a ect the depletion interaction. Croze and Cates suggested that there is a nonadditive property between the electrostatic interaction and the depletion interaction [2]. Our aim of this study, therefore, is to give experimental information on the structure of protein solutions with polymers while controlling the electrostatic interactions with added salt.

The structure of protein solutions in this study were measured by small angle x-ray scattering with a synchrotron-radiation light source (SPring-8). Glucose isomerase was used as a model protein. The pH of the solution was controlled with 25 mM phosphate bu er, which gives the minimum salt concentration (The molar concentration of the protein was much lower than that of the bu er, so was that of the counter ions). Polyethylene glycol, PEG (Fluka) was used as a soluble polymer, whose molecular weight was 10,000 (g/mol).

Figure 1 shows the intensity pro les for the glucose isomerase solutions without PEG (a) and with PEG 10%(w/v) (b). The protein concentration was kept at 3.0%(w/v). NaCl was added upto 1.0 M. The solvent scattering was subtracted from the raw data and then they were divided by the absorbance of the solvent. The scattering from PEG or NaCl was found to be signi cantly lower than that from the protein.

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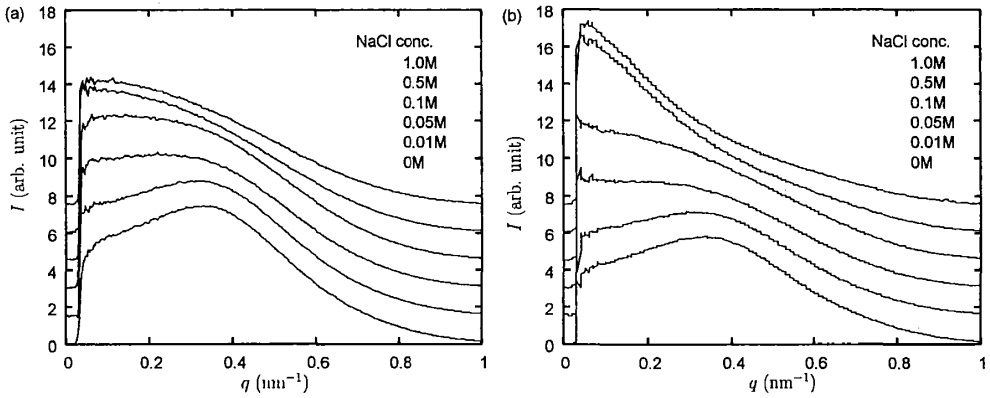

Figure 1: Intensity profiles for glucose isomerase solutions without PEG (a), and with PEG 10%(w/v) (b). The protein concentration was kept at 3.0%(w/v).The base lines of the profiles are shifted to facilitate visualization.

In g. 1a, the intensity in low q region was suppressed when NaCl concentration was low because of the electrostatic repulsion. This suppression was removed gradually with the added NaCl. However, no upturn of the pro le in low q region was observed even at the highest concentration of NaCl. This suggests that the added NaCl screened the electrostatic interaction but did not induce any attraction since the upturn of the pro les is the signature of the particle attraction. On the other hand, the pro les showed the clear upturn when PEG was added in the solutions (g. 1b), which indicates the PEG-induced attraction between proteins. Interestingly, this attraction was not observed at all when the electrostatic interaction was strong (at 0-0.05 M of NaCl). In the low-salt solutions, the electrostatic interaction seems completely dominate the PEG-induced attraction.

To discuss the interparticle interaction quantitatively in some measure, we conducted a Monte Carlo simulation with the e ective interaction potential $U(r)$, and compared the results with the experiments. Brie y, an e ective potential which consists the screened Coulomb and a constant model attraction for PEG-induced potential can explain the experimental results. In the presentation we will discuss this in detail.

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References

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