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Relation between the depletion interaction and the electrostatic interaction in protein solutions

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1 Introduction

In protein crystallization, polyethylene glycol (PEG) is one of the most popular and successful reagents. It has been proposed that the depletion mechanism often used for colloid-polymer mixtures is the possible source of attractive interactions induced by PEG between protein molecules [1-3]. The fact that the attraction strongly depends molecular weight of PEG supports the depletion mechanism.

In addition to PEG, salt such as NaCl, is the another important reagent for the protein crystallization. The electrostatic repulsion between protein molecules must be screened by ionic reagents. In fact, the combination of PEG and salt is frequently used to produce protein crystals and marks high success rate, although it remains unclear how these two types of reagents work together in protein solutions.

In this study, we are interested in the competition between the depletion interaction and the electrostatic interaction acting on protein molecules, and how the crystallization reagents such as PEG and NaCl work to induce a net attraction between them.

2 Experiments

The dynamic light scattering (DLS) was used to quantitatively measure the interaction between protein molecules. The collective diffusion coefficient $D$ obtained by DLS is the function of the volume fraction of proteins $\phi$, and is approximated as

$$D = D_0(1 + \lambda\phi),$$

where $D_0$ is the diffusion coefficient in the dilute limit and $\lambda$ is the interaction parameter. We used a protein glucose isomerase as a model system. The glucose isomerase, whose radius is $1$
about 4 nm, is a tetramer composed of four identical polypeptide of molecular weight 43000. We measure \( \lambda \) of the protein solutions with or without the crystallization reagents, NaCl and PEG and see how they affect the interaction between protein molecules.

3 Results and discussion

Figure 1 shows the interaction parameter \( \lambda \) versus the PEG concentration \( c_{\text{PEG}} \). Without NaCl (solid symbols), \( \lambda \) was almost independent of \( c_{\text{PEG}} \) within the experimental error, whereas with NaCl (open symbols), \( \lambda \) decreases with \( c_{\text{PEG}} \) from a positive value to negative one. These results indicate that PEG and NaCl work coherently in order to introduce attraction between protein molecules. This is why PEG is widely used with salt for protein crystallization.

Our crude scenario for the mechanism of this cooperative effect of PEG and NaCl is as follows. Glucose isomerase has net negative charges on the surface. Therefore, there are strong electrostatic repulsion between molecules. Although the solution contains counter ions as well as buffer ions (sodium phosphate, 25 mM), the Debye screening length without NaCl is rather long, estimated about 1.6 nm. On the other hand, the depletion works only when the two 'depletion zone', where a part of polymers are prohibited to enter, overlap. This length is roughly approximated as the radius of gyration of PEG, which is in the range of a few nm. Therefore, if two protein molecules cannot approach each other close enough to overlap the two depletion zones, the depletion attraction would not be induced. In other words, the depletion is switched off if repulsion between the particles is too strong.

In the presentation, we will discuss in detail our experimental results in addition to some Monte Carlo simulation results, where proteins and polymers are modeled by two different spheres.

References