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Small angle X-ray scattering of an artificial four helix bundle protein

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1 Introduction

Creating water-soluble proteins has been one of the challenging issues in a protein design research because of lack of knowledge about a protein self-interaction and a solvation mechanism. We have developed a method for extracting the protein self-interaction using small angle X-ray scattering (SAXS) and a liquid state theory [1]. To extend the study, we have targeted natural proteins such as lysozyme and artificial proteins. The former is for determining the self-interaction potential, by which the natural proteins avoid the aggregation or precipitation. The latter is for getting insights of what kinds of design are effective for making the artificial proteins soluble; yet, concentrating the artificial proteins for analysis of the interaction often resulted in precipitation so far.

On the other hand, characterizing solution structures of the artificial proteins is beneficial for improving the structure and the stability. SAXS analysis is preferred because it is often bottlenecking to crystallize proteins. Accordingly, we have conducted SAXS measurement for the artificial proteins designed by Isogai *et al*. (e.g., [2]).

2 Experiment

The amino acid sequence of the protein, bHH is HMLKKLREEHLKLLEEFKKLLEEHLKWLEGGGGG GGGELLKLHEELLKKFEELLKLHEERLKKL, which was synthesized by using *E. coli* and purified as previously described [2]. The SAXS experiment was performed at the beamline BL-10C. The X-ray wavelength, λ, was 0.1488 nm; the camera length was 1049 mm, which was calibrated by use of a scattering pattern of silver behenate. X-ray intensities were recorded by PILATUS3 2M (DECTRIS Ltd., Switzerland). bHH was dialyzed against the aqueous solution with 10 mM HEPES-NaOH and 50 mM NaCl (pH 7.2). The protein concentration was 2.8 mg/mL. After centrifugation, the supernatant solution was measured. 150 images were collected; an image was recorded for 2 sec of the exposure time. The temperature was controlled to be 10 °C. The circular 1D average of the image was performed by the program *Nika* [3]. The 1D data were averaged; damage by radiation was negligibly small. The scattering parameter q is defined as $q = 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle of X-rays.

3 Results and Discussion

Fig. 1 shows the SAXS profile of the bHH protein. Guinier analysis of the profile in the q region of 0.016–0.045 Å^{-1} gave 25.5 ± 0.1 Å of the radius of gyration (Rg). A fitting analysis using a theoretical scattering function indicates that the cylinder [4] with a radius of 14.6 ± 0.3 Å and the length of 77.9 ± 0.8 Å was a good approximation for the experimental scattering as shown in Fig. 1. According to the previous study [2], the two chains of bHH were associated, where the single chain of bHH formed a helix–loop–helix structure: the bHH was a four helix bundled. The given radius (14.6 Å) of the cylinder model for bHH was in agreement with that expected; we estimated the radius to be ~14 Å using the crystal structure of the other four helix bundle protein (PDB: 1M3W). On the other hand, the given length (77.9 Å) was longer than that expected (<~50 Å); it is assumed that a helical turn has a pitch of 5.4 Å consisting 3.6 residues. This implies that the misaligned helix bundled molecules or further associated molecules would be contaminating.

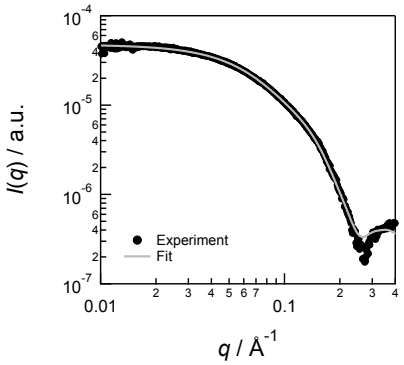

Fig. 1: Small angle X-ray scattering profile of the artificial protein, bHH (black filled circles). Grayline represents the best fit curve calculated based on a cylinder model.

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References

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