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Src SH3 forms unfolded α-helix rich conformation in 15% trifuoroethanol

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Introduction

Src SH3 domain protein is a small fully β-sheeted protein of 57 residues. We have found that the protein changed its conformation to the one with higher α-helical content in trifluoroethanol (TFE) of which mixing ratio against water was higher than 15%, judging from CD measurement. In the present study we have measured the conformation of the preotein by x-ray solution scattering at various TFE concentration.

Experimental

X-ray scattering experiments were done at the beamline of 15A, keeping the sample-to-detector-distance at c.a. 1.3 m with a CCD-based X-ray detector (Hamamatsu Photonics, C7300). The obtained data were corrected for distortion of images, non-uniformity of sensitivity, and the contrast reduction for and X-ray image intensifier.

Src SH3 was dissolved in the phosphate buffer of pH 6 in the presence and absence of TFE. Temperature was kept at 4±0.1ºC. In the experiments, TFE is mixed with the phosphate buffer. The concentration of TFE was determined as v/v ratio against the buffer solution.

Results and Discussion

Fig.1 shows the radius of gyration (Rg) of src SH3. In TFE condition (5-15% TFE), Rg values are slightly larger than that of the native state (0% TFE). Kratky plots are shown in Fig.2. Src SH3forms compact state in 0 to 10% TFE. In contrast, at 15% TFE, the Kratky plot shows gradual increase with no peaks, indicating the protein is not compact. However, Rg is not so large as that of the fully unfolded state (c.a. 28Å). This conformation is probably different from the transient α-helix-rich intermediate observed on the refolding pathway of the protein [1]. We, then, calculated the molecular shape of src SH3 in 15% TFE concentration by GASBOR[2] program. The obtained structure is shown in Fig.3. It looks partially unfolded.

In higher TFE concentration region such as 30% or 40%, water/TFE mixtures show strong scattering. This indicates TFE molecules form some structures in the solvent, which disturbed the precise analysis of protein structure seriously.

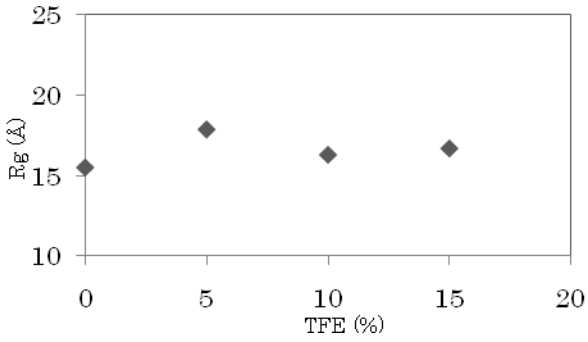

Fig. 1 Rg of src SH3 against TFE/buffer (v/v)

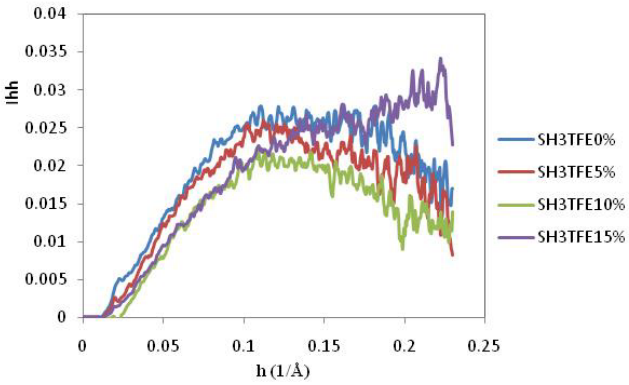

Fig. 2 Kratky plots of src SH3 in 0-15% TFE

Fig. 3 Reconstructed shape of src SH3 in the presence of 15% TFE calculated by GASBOR [2]

References

[1] Li *et al.* (2007) Biochemistry, 46, 5072-5082.
[2] Svergun *et al.* Biophys. J, 80, 2946-2953 (2001).

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