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Refolding of a mutant, A45G, of src SH3 at pH 3

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Introduction

Src SH3 domain protein is a small single full β-sheet protein of 57 residues. We have found that the src SH3 formed an α-helix-rich transient intermediate (TI) on its folding pathway between pH 3 and 6 [1]. A mutant, A45G, of src SH3 was also folded via. an α-helix-rich TI at pH6. In addition, A45G formed an α-helix-rich equilibrium intermediate (EI) at pH3, though it forms the native conformation at pH 6 in equilibrium [2]. It is, then, of interest to investigate TI and EI comparatively.

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.

On refolding, A45G, which was dissolved in 5 M GuHCl with 50 mM phosphate buffer, was mixed with 7 volumes of 50 mM phosphate buffer. Thus the GuHCl was diluted to 0.7 M. The refolding was monitored by x-ray scattering kinetically. Final protein concentration was 0.5 mg/ml. Temperature was controlled at 4°C.

Results and Discussion

We have repeated the same experiments three times. Each time, radius of gyration (Rg) did not show significant dependence on time. In Fig. 1, the averaged Rg of the three experiments is shown against time. It shows a flat line, which is so different from the single exponential changes of wild SH3 at pH's 3 and 6 and of A45G at pH 6 [1, 2]. The averaged Rg value is 20.2Å.

Rg values of wt SH3 and A45G so far obtained in equilibrium are summarized in Table. 1. Rg values of the native conformation (both pH 3 and 6) and that of A45G at pH 6 are nearly the same (15.2±1Å). Rg values of the unfolded state are 27.5±0.5Å, and Rg value of A45G at pH 3 is 19.1Å.

Rg value of TI was obtained as 19Å [1]. Thus, Rg values of TI of wt SH3, EI of A45G at pH 3 are the same with Rg values obtained in the present study as shown in Fig. 1 within experimental errors.

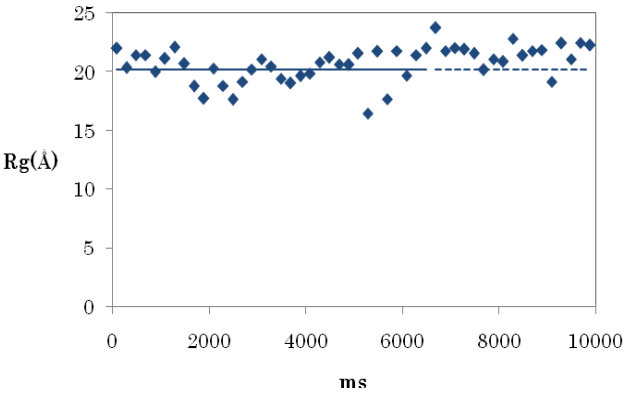

Fig. 1. The averaged Rg of three runs of A45G refolding at 4 °C and at pH3.

Protein	Condition	*Rg* (Å)
A45G	pH 6.0	15.4
	pH 4.0	17.3
	pH 3.0	19.1
	5M GuHCl	28
WT	pH 6.0	15.1
	pH 4.0	14.8
	pH 3.0	15.3
	5M GuHCl	27

References

[1] Li *et al.* (2007) Biochemistry, 46, 5072-5082.
[2] Li *et al.* (2007) J. Mol. Biol., 372, 747-755.

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