

Time-resolved kinetic refolding of PI3K SH3 in the presence of 45% ethylene glycol at pH 6

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Introduction

We have investigated kinetic protein folding study of src SH3 domain and its mutant, A45G and Fyn SH3 domain, by various probes including X-ray solution scattering combined with cryo-stopped-flow method [1, 2]. We have done kinetic refolding experiments of another SH3 domain, PI3K SH3 domain. This protein is also mainly composed of β -sheets as src SH3 domain [3]. In the present study, we performed kinetic refolding of PI3K SH3 domain by a concentration jump of guanidine hydrochloride (GuHCl) from 5 M to 0.7 M. The experiments were done in the presence of 45% ethylene glycol at pH 6 at -5°C .

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 image distortion, non-uniformity of sensitivity, and the contrast reduction on X-ray image intensifier.

Results and Discussion

Figure 1 shows kinetic refolding of PI3K SH3 domain of time-resolved SAXS measurements by the stopped-flow in the presence of 45% ethylene glycol at pH 6 at -5°C . The figure does not show time-dependent change on R_g within the experimental errors. The averaged R_g value of the fitting line was $17.7 \pm 1.0 \text{ \AA}$. The R_g of the native state of the PI3K SH3 domain is 15 \AA . In contrast, the R_g of the unfolded state of the protein is 27.5 \AA . Dobson *et al* reported that the slow refolding took place with rate of 0.0565 and 0.0140 s^{-1} [4]. Taken into consideration of these native and denatured R_g values, it is thought that the obtained R_g value we observed is a kinetic transient intermediate, of which size is slightly bigger than the native structure.

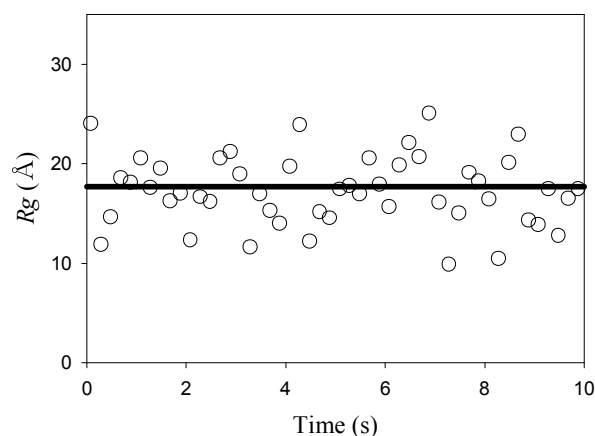


Fig. 1 Kinetic refolding of PI3K SH3 domain of time-resolved SAXS measurements by the stopped-flow in the presence of 45% ethylene glycol at pH 6 at -5°C . The black line is fitting line. The R_g was 17.7 \AA .

References

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