

Unfolding of PI3K SH3 in the presence of 45% ethylene glycol at pH 6

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Introduction

Previously, we have reported thermodynamic and kinetic studies of src SH3 domain and its mutant, A45G, by various probes including X-ray solution scattering [1, 2]. We have started the study on 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 guanidine-hydrochloride (GuHCl) titration of PI3K SH3 domain in the presence of 45% ethylene glycol at pH 6 at 4°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.

Concentration of PI3K SH3 domain was 1 to 2 mg/mL.

Results and Discussion

Figure 1 shows GuHCl concentration dependence of R_g of PI3K SH3 domain in the presence of 45% ethylene glycol at pH 6 at 4°C. R_g values from 0 M to 2 M GuHCl were almost constant. The average value of the R_g (except 1 M) was 15.3 ± 0.3 Å. This value is in good agreement with that of native state of src SH3 domain [1]. This indicates that PI3K SH3 domain takes native state at least until 2 M at this condition. In contrast, R_g values from 3 M to 5 M GuHCl were around 30 Å. The average value of the R_g was 27.5 ± 0.7 Å. This R_g value also is very similar with that of the unfolded state of src SH3 domain [1]. This suggests that PI3K SH3 domain is denatured at least higher than 3 M GuHCl concentration.

Figure 2 shows Kratky plot of PI3K SH3 domain in the presence of 45% ethylene glycol at pH 6 at 4°C (= 0 M GuHCl). It is clear that the Kratky plot has obvious one peak. This indicates that the native state of PI3K SH3 domain is compact globule state.

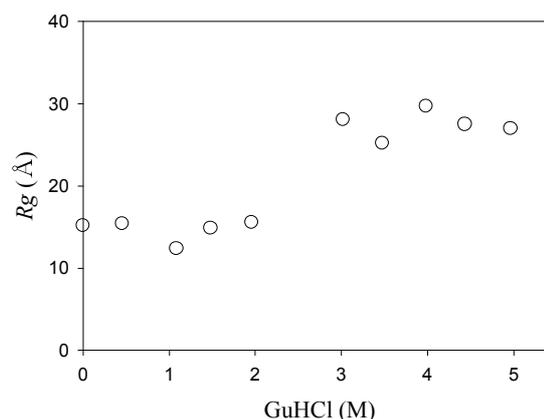


Fig. 1. GuHCl concentration dependence of R_g of PI3K SH3 domain in the presence of 45% ethylene glycol at pH 6 at 4°C.

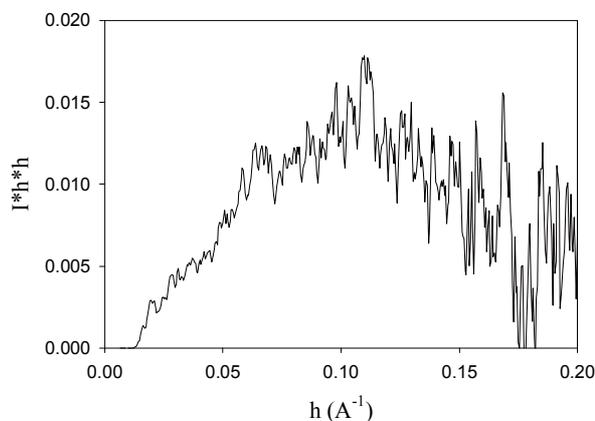


Fig. 2. Kratky plot of PI3K SH3 domain in the presence of 45% ethylene glycol at pH 6 at 4°C.

References

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