

Guanidine hydrochloride-induced unfolding transition of pseudo-WT Fyn SH3 at pH 6 in the presence of 45 % ethylene glycol at 4 °C

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Introduction

Fyn SH3 is a small β -sheet protein, composing of five β -strands with a molecular weight of 9,300. It is homologous to src SH3 (homology of their amino acid sequences is 73 %). As their native conformations are very similar, it is of interest to compare thermodynamic stability of them. Thermodynamic study of Fyn SH3 with its mutants has been studied [1].

In the present study, we performed guanidine-hydrochloride (GuHCl) titration of pseudo-wild-type Fyn SH3 (A39V, V55F) in the presence of 45% ethylene glycol at 4°C.

Results

Fig 1 shows Guinier plots of the native and the denatured state of the pseudo-WT Fyn SH3. Radius of gyration (R_g) of the native pseudo-WT Fyn SH3 is 15.9 ± 0.5 Å, whereas R_g of the denatured pseudo-WT Fyn SH3 is 27.2 ± 1.9 Å. These values are in good agreement with src SH3 domain that we have reported (14.6 Å at the native state and 26.7 Å at the denatured state) [2].

Fig 2 shows GuHCl concentration dependence of R_g . As seen in Fig 2, R_g of the pseudo-WT Fyn SH3 was nearly constant below 2 M, and become bigger with the increase of GuHCl concentration upto 3.5 M. Then, R_g is constant above 3.5 M.

Kratky plots of the native and the denatured pseudo-WT Fyn SH3 are shown in Fig 3. It is clear that the native state has one peak, whereas that of the denatured state is broad over the large angle region. This shows that the native state of pseudo-WT Fyn SH3 is compact globule state and the denatured state is unfolded.

From the present study, it is clear that pseudo-WT Fyn SH3 is changed from the folded state to the unfolded state with the addition of GuHCl in the presence of 45 % ethylene glycol.

References

- [1] K. L. Maxwell *et al.*, *Biochemistry*, **37**, 16172 (1998).
 [2] J. Li *et al.*, *Biochemistry*, **46**, 5072 (2007).

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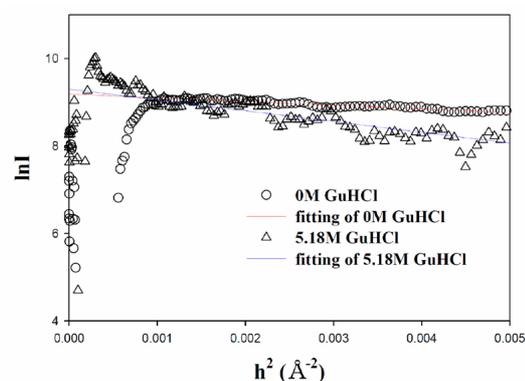


Fig 1. Guinier plots of native and denatured state of pseudo-WT Fyn SH3 at pH 6 in the presence of 45 % ethylene glycol. 4°C

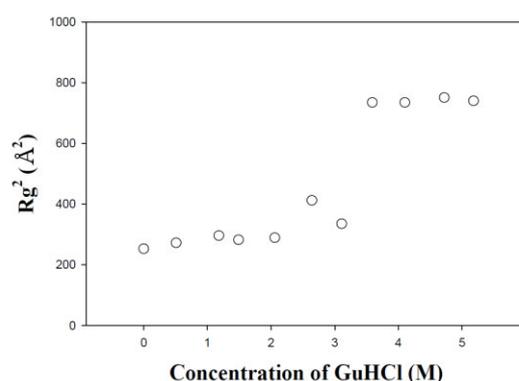


Fig 2. GuHCl concentration dependence of R_g of pseudo-WT Fyn SH3 at pH 6 in the presence of 45 % ethylene glycol. 4°C

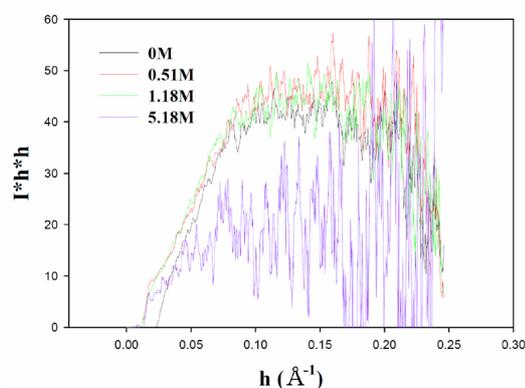


Fig 3. Kratky plots of native and unfolded state of pseudo-WT Fyn SH3 at pH 6 in the presence of 45 % ethylene glycol. 4°C