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Src SH3 mutant, A45g, takes a molten globule state at pH 3

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Introduction

Src SH3 is a β-sheet protein, of which molecular weight is 8,700. It forms 5 β-strands at pH 3 as well as at pH6. In contrast, a mutant (A45G) takes α-helix-rich conformation at pH3, while it takes 5 β-strands at pH 6 [1]. It is then of interest to investigate conformation of A45G at pH3 by solution x-ray scattering.

Results

A45G at pH3 was measured by solution x-ray scattering method installed at beam-line 15A. Figure 1 shows a protein concentration dependence of obtained Rg. As seen in the Figure, Rg is nearly constant. By the extrapolation to zero concentration, Rg was obtained as 19.5 Åslightly higher than that at pH 6, 14.6 Å[2]. Kratky plot of the A45G at pH3 shows a peak. From these findings, we can conclude A45G takes a molten globule state at pH 3.

As a next, unfolding of A45G was measured as a function of urea. In Fig. 2, concentration dependence of Rg^2 on urea is shown. A45G was unfolded with the mid-point at 2.5M urea, taking Rg of 26.7Å at high urea concentration. In Fig. 3, typical Kratky plots are shown. The figure demonstrates A45G takes globular structure in the absence of urea, and unfolded above 4 M urea.

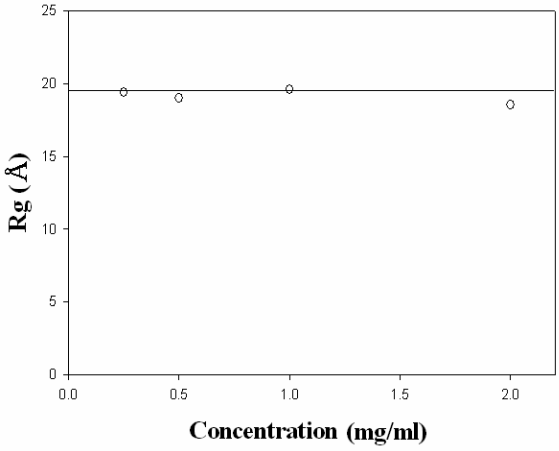

Figure1.Rg of A45G at pH 3, 4ºC. Protein conenctration dependence

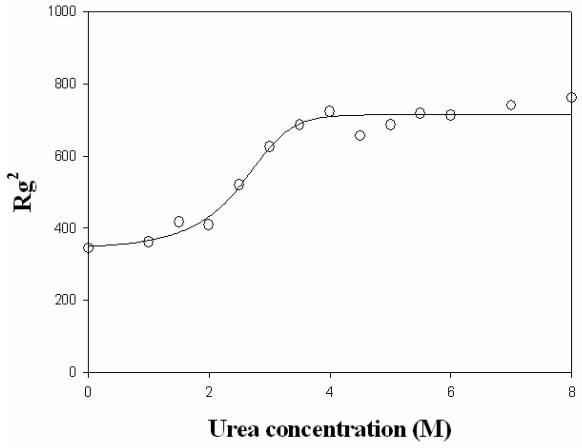

Fig. 2. Rg of A45G at pH3. Urea titration.

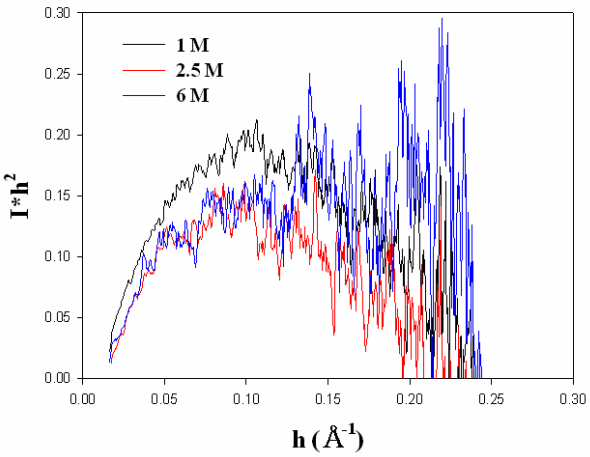

Fig.3.Kratky plot of A45G

References

[1] Li *et al*. J. Mol. Boil., in press..
[2] Li *et al*. (2007) Biochemistry, 46, 5072-5082.

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