

α -Helix-rich intermediate of all β -sheet protein, src SH3 at acidic pH

Jinsong LI, Masaji SHINJO, Yoshitaka MATSUMURA, Hiroshi KIHARA*

Department of Physics, Kansai Medical University, 18-89 Uyama-Higashi, Hirakata 573-1136, Japan

Introduction

The small all- β -sheet protein, src SH3, was found to take α -helix-rich intermediate on the folding pathway [1, 2]. To investigate the intermediate in more detail, we started mutating the protein, and surprisingly we found that single mutation of src SH3, A45G, formed α -helix-rich conformation at acid condition. Secondary structure analysis indicated kinetic intermediate has a similar secondary structure composition with acid-induced intermediate.

The purpose of this study is to characterize acid-induced intermediate by x-ray solution scattering.

Result

Guinier analysis: The x-ray solution scattering of pseudo src SH3 (A45G) was performed at different pH in 50mM PBS buffer at 4°C. Radius of gyration (Rg) was calculated both from Guinier plots in the region of $h \leq 0.05$, where the value of $R_g \cdot h$ is less than 1 ($h = 4\pi \sin\theta / \lambda$, λ is the wavelength (1.5Å) and θ is the half of the scattering angle). The Rg of A45G at pH 6.0 is 15.38Å, very close to Rg of the native state of WT. But at pH 4.0, the Rg increased to 17.37Å, indicating the protein was slightly expanded. The Rg's estimated from Guinier approximation and Kratky plot were summarized in Table1.

Table1. Radius of gyration of src SH3 at different pH

Protein	Condition	Rg from Guinier plot	Rg from Kratky plot
A45G	pH 6.0	15.38	13.8
A45G	pH 4.0	17.37	16.2
A45G	pH 3.0	18.39	18.2
A45G	pH 1.5	30.9	
WT	pH 6.0	15.1	

At pH 1.5, the Guinier approximation cannot give a precise estimation of Rg because $\ln(I) \text{ vs } h^2$ does not show linear relation at Guinier region. This indicates the protein was associated at acid pH.

Kratky plot analysis: As well known, the Kratky plot can be used to judge the conformation of protein in solution. To judge the conformation of A45G at different pH, we also made Kratky plots (Fig. 1). It is clear that the protein is compact at pH 6.0, 4.0 and 3.0 as the curves show clear peaks, respectively. It is also useful to estimate the Rg from its peak position. The results are also summarized in Table1. At pH 1.5, the Kratky plot shows no peaks due to strong association. At pH 3.0,

there also observed a shoulder at very small angle region, indicating the association. Rg obtained from Guinier plots and Kratky plots are in good agreement.

The Rg of A45G at pH 3.0 is about 1.2 times larger than the native state (pH 6.0). This indicates SH3 is in the molten globule state at acid condition.

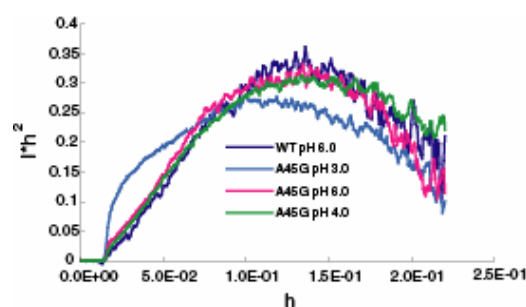


Fig 1 Kratky plot of src SH3 at various pH

References

- [1] Qin et al., manuscript in preparation
- [2] Li et al., manuscript in preparation.

kihara@makino.kmu.ac.jp