15A/2004G184

Kinetic intermediate on src SH3 folding pathway investigated by SAXS

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Introduction

Src homology SH3 domain is a small protein (50~70 amino acids), which is involved in the signal transduction in the cell. We have found a α-helix-rich intermediate on its folding pathway by SF-CD, though conventional secondary structure prediction methods indicate very low helix propensity in its primary sequence. The α-helix-rich intermediate was formed very fast within the dead time of SF (6ms). Elucidation of the α-helix-rich intermediate conformation is important to help us understand the folding mechanism of β-sheet protein.

Here, we performed refolding src SH3 by SAXS at the same condition with SF-CD.

Result

X-ray scattering data were analyzed by Guinier analysis. The linear Guinier region was selected to obtain radius of gyration (R_g) in the region of $h \leq 0.03$, where the value of $Rg*h$ is less than 1

Rg's thus obtained are plotted as a function of GuHCl in Fig 1. Rg of the protein at the native state was 14.6 Å, similar to that calculated from its crystal structure. With the increase of GuHCl, Rg increased to 27 Å at 5M GuHCl, indicating the protein was totally unfolded at 5M GuHCl.

Figure 2 shows the kinetic refolding curve of src SH3 in terms of radius of gyration (Rg). The figure shows a burst phase and an observable phase. The Rg of the protein dramatically decreased from 27 Å (Rg of the unfolded src SH3 at 5M GuHCl) to 18.1 Å within the dead time of SF. Then it changed to 15.1 Å with a single exponential decay with the rate of 0.94 s^{-1}. The final value of Rg of this observable phase is in good agreement with that of the native state (14.6 Å).

Figure 3 shows Kratky plot of the refolding process of src SH3. where $h=4\pi\sin\theta/\lambda$ and θ is the half of the scattering angle. The src SH3 was already compact within the dead time of the stopped flow, though it was different from the refolded state (5s later after the start of the refolding). Furthermore, the peak position of the intermediate curve appeared at the smaller angle, comparing with the refolded curve. This indicates compactness of the intermediate is not as tightly packed as the refolded. Rg, calculated from Kratky plots, is similar to Rg estimated from Guinier approximation. Therefore, the process of src SH3 becoming compact took place clearly via. two steps, and it is again in good agreement with the observation of SF-CD and SF-fluorescence.
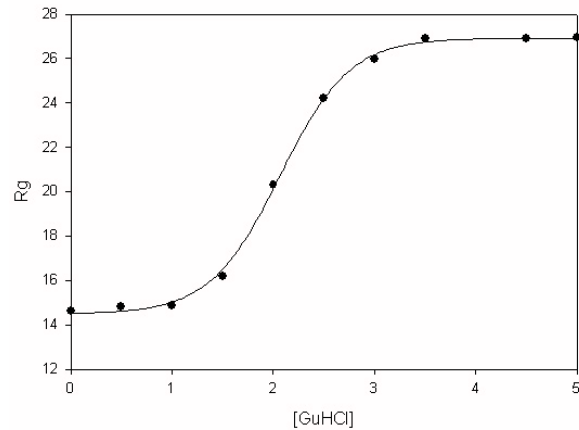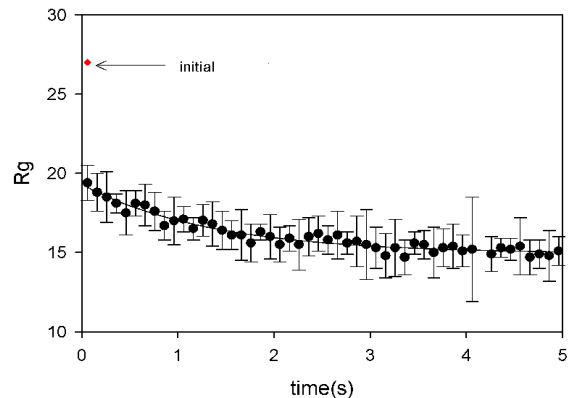

Fig 1 GuHCl-induced unfolding of src SH3

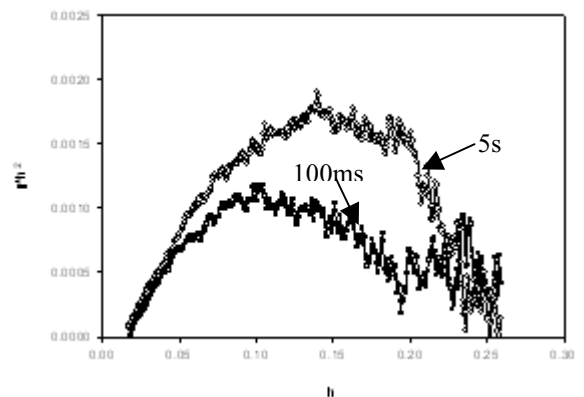

Fig 2 Time course of Rg of SH3 refolding

Fig 3 Kratky plot of SH3 at different time frame

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