Biological Science

15A/2004G184

Time resolved SAXS study of SH3 protein refolding

Jinsong Li, Masaji Shinjo, Yoshitaka Matsumura, Hiroshi Kihara*
Department of Physics, Kansai Medical University, Hirakata 573-1136, Japan

Introduction

Src SH3 is a β-rich protein without any α-helix in equilibrium. However when we performed refolding experiment of the protein monitored by circular dichroism, big α-helical burst was observed within the dead time of the stopped-flow apparatus (6 ms), which was followed with the conversion of α-helix to β-sheets with the rate of a few sec at 4 °C

To elucidate the conformation of the initial burst phase, we performed SAXS experiment at the same condition.

Results

First, we performed x-ray scattering of src SH3 at pH3 and 4 °C by changing the concentration of the denaturant, GuHCl. The protein takes unfolded state with the mid point at 2.0 M of GuHCl. Radius of gyrations (Rg) at the native state and the unfolded state are 14.6Å and 26.9Å,respectively.

Time-resolved refolding experiment was also done at pH 3 and 4°C. One volume of Src SH3 in 5mol/l GuHCl was mixed with six volumes of PBS buffer with the stopped flow apparatus. Time slices were taken at each 100 ms.

In Fig. 1, Kratky plots at various time flames are shown, indicating the change from the burst phase to the native state. Rg's at each time slice were estimated from Guinier plot, and are shown in Fig. 2. It changes from 18.5Å to 15.3Å with the rate of 1.4 s^{-1}.

The Rg of the first point (50 ms after the mixing) is 18.5Å, significantly smaller than the value of the unfolding state, 26.9Å. This demonstrates that the protein is packed to the intermediate which is much more compact than the unfolded state, while Rg at 5s was almost similar to the folded Rg (14.6Å). I_0's were also obtained and plotted in Fig. 3, showing no changes through the refolding process. This shows there are no associations occurred during the refolding process.

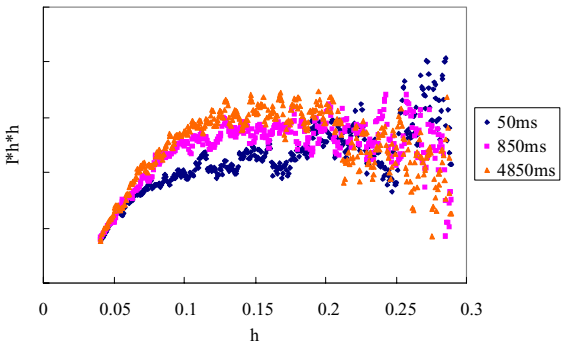

Fig. 1. Kratky plot of SH3 at various time.

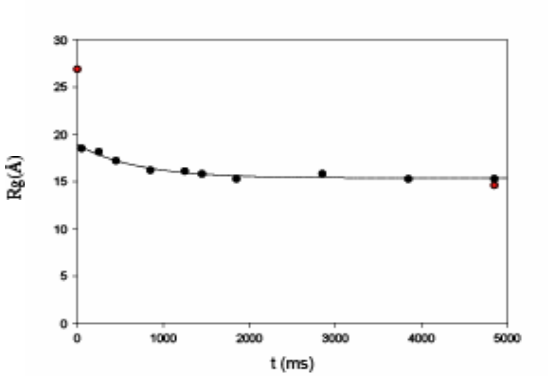

Fig2 . Rg (Å) vs. t(ms). .Red points represent Rg observed in equilibrium.

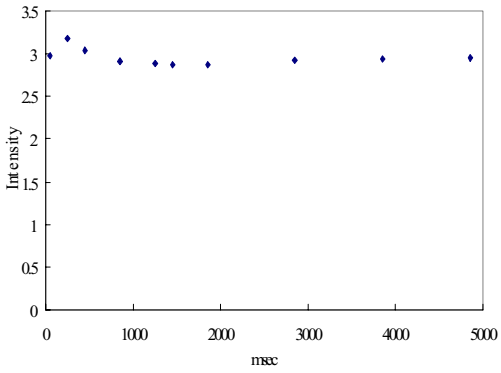

Fig3. I_0 vs. t(ms).

* kihara@makino.kmu.ac.jp