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Time-resolved SAXS on FHA1 domain of Rad53 refolding at cryo conditions

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Introduction

FHA1 domain of Rad53 is composed 151 amino acid residues and consists of 11 β-strands; β1 ~ β10 are anti-parallel, β3 ~ β3' are parallel β-strands, and 3 α-helices. The shape of FHA1 domain of Rad53 is a β-sandwich typed, which is formed by two large twisted anti-parallel β-sheets [1, 2].

We carried out kinetic refolding experiments of FHA1 domain of Rad53 at subzero temperature by SAXS combined with cryo-stopped-flow (SF) method.

Results

Fig 1 shows Guinier plots of native and unfolded states of FHA1 domain of Rad53 in the presence of 45% ethylene glycol (EGOH) at 4°C *Rg* values of the native and the denatured states were obtained as 21.2 ± 0.4 Å and 35.5 ± 1.6 Å, respectively. From Kratky plots, the native state of the protein has a peak, whereas the denatured state has no peaks (data not shown). These results indicate that the native state of the protein was compact and globule, and the unfolded state was not.

We performed kinetic refolding experiments of FHA1 domain of Rad53 in the presence of 45% EGOH at -20°C by SAXS combined with cryo-SF method. *Rg* of time dependence of the kinetic refolding process was shown in Fig 2. Although the data in the figure is noisy, we could obtain refolding time course and a single exponential fitting curve of the refolding data (solid curve in Fig 2). There are two phases, a burst phase and an observable phase. The *Rg* of FHA1 domain of Rad53 was largely decreased from 35.5 Å to 27 Å within the dead time of the stopped-flow apparatus (= burst phase). Then, *Rg* of the refolding trace was decreased with a single exponential decay (= observable phase). The *Rg* of the burst phase was 27.0 Å. This value is larger than of the native state (21.2 Å), and smaller than the denatured state (35.5 Å). This suggests that FHA1 domain of Rad53 forms the transient kinetic refolding intermediate, the intermediate of the protein was compact and its molecular size is bigger than the native structure (21.2 Å).

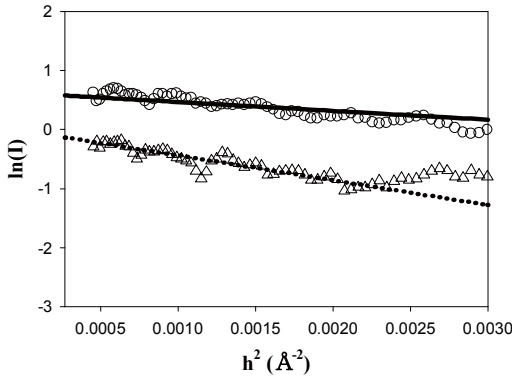

Fig 1. Guinier plots of native (circle) and denatured state (triangle) of FHA1 domain of Rad53 in 50 mM phosphate buffer at pH 6.5 in the presence of 45% EGOH at 4°C.

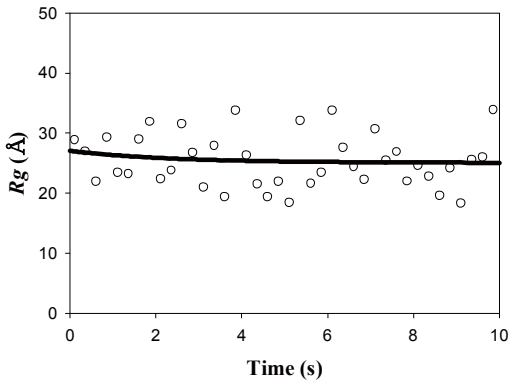

Fig 2. Kinetic refolding of FHA1 domain of Rad53 of time-resolved SAXS measurements in 50 mM phosphate buffer at pH 6.5 in the presence of 45% EGOH at -20°C by SAXS combined with cryo-stopped-flow. The solid curve is a single exponential fitting curve. The rate constant was 0.42 ± 1.2 s^{-1}.

References

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