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Time-resolved SAXS on Ki67 FHA domain refolding at cryo conditions

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Introduction

Human Ki67 FHA domain is composed 120 amino acid residues and consists of β-sheets and no helices [1]. This protein is β-sandwich typed. Human Ki67 protein is involved in the protein interaction network that drives cell division cycles, which contains a FHA domain [1].

We investigated the molecular size of the protein in native and denatured states at cryo conditions. We also performed kinetic refolding experiments of human Ki67 FHA domain at subzero temperature by SAXS combined with cryo-stopped-flow (SF) method.

Results

Fig 1 shows Guinier plots of native and unfolded states of human Ki67 FHA domain in 50 mM phosphate buffer at pH 7.5 in the presence of 45% EGOH and 2 mM DTT at 4°C in equilibrium. *Rg* values of the native and the denatured states were obtained as 15.9 ± 1.8 Å and 26.5 ± 1.2 Å, respectively.

We also investigated kinetic refolding process of human Ki67 FHA domain at subzero temperature by SAXS combined with cryo-SF method. The cryo conditions were in 50 mM phosphate buffer at pH 7.5 in the presence of 45% EGOH and 2 mM DTT at -28°C. The result of the kinetic refolding experiments of the human Ki67 FHA domain is shown in Fig 2. The figure shows the time-resolved *Rg* change. From the figure, the refolding trace has no time course within the experimental error. The averaged *Rg* value of the fitting line (solid line in Fig 2) was 18.2 ± 0.5 Å. This value is slightly bigger than the native structure and much smaller than the unfolded state. It suggests that Ki67 FHA domain forms the transient kinetic refolding intermediate and its molecular size is compact.

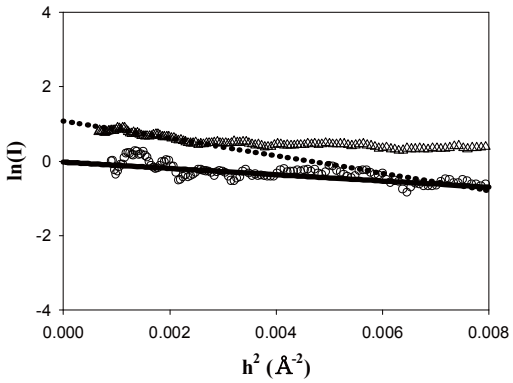

Fig 1. Guinier plots of native (circle) and denatured state (triangle) of Ki67 FHA domain in 50 mM phosphate buffer at pH 7.5 in the presence of 45% EGOH and 2 mM DTT at 4°C.

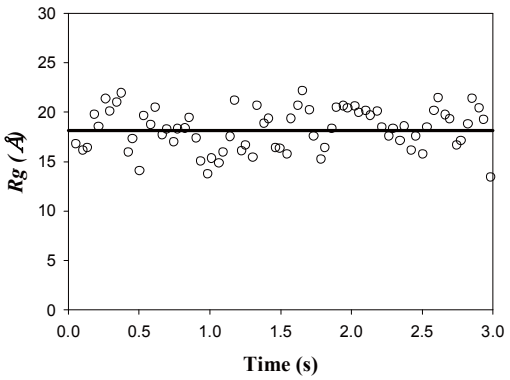

Fig 2. Kinetic refolding of Ki67FHA domain of time-resolved SAXS measurements in 50 mM phosphate buffer at pH 7.5 in the presence of 45% EGOH and 2 mM DTT at -28°C by cryo-stopped-flow. The solid line is the fitting line. The *Rg* was 18.2 ± 0.5 Å from the fitting.

References

[1] H. Li et al., (2004) J. Mol. Biol., 335, 371-381.

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