

# $\alpha$ -Helical burst on the folding pathway of FHA domains from Rad53 and Ki67

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We have been studying folding of  $\beta$  proteins (src SH3, its mutant A45G, bovine and equine  $\beta$ -lactoglobulin). They showed  $\alpha$ -helix-rich intermediates at the earliest stage on the folding pathways.

We have a simple question: do other  $\beta$  proteins also form  $\alpha$ -helical intermediates on their folding pathway? So, we have studied other  $\beta$  protein, FHA domains.

We did refolding experiments by cryo-stopped-flow CD. The result was that FHA1 domain of Rad53 showed three phases (I, II and III) at  $-20^{\circ}\text{C}$ . The phase I is the burst phase with the increase of  $\alpha$ -helix. The phase II is observable (0.25 s<sup>-1</sup>) phase, in which  $\alpha$ -helix decreased. The phase III is slower than 30 min. When temperature increased to  $-15^{\circ}\text{C}$ , the phase II became too fast to be detected. Actually, the CD value of the burst phase of  $-15^{\circ}\text{C}$  is similar to the CD value of the last level of the phase II of  $-20^{\circ}\text{C}$ . Instead, another phase ( $\alpha$ -helix decrease) appeared at  $-15^{\circ}\text{C}$ . Judging from its CD value, it seems that the observable phase of  $-15^{\circ}\text{C}$  corresponds to the phase III of  $-20^{\circ}\text{C}$ . We also measured time-resolved SAXS at  $-20^{\circ}\text{C}$ . From the  $R_g$  values, it is thought that  $\alpha$ -helical burst intermediates at  $-15^{\circ}\text{C}$  and  $-20^{\circ}\text{C}$  (phase I and phase II) were compact.

Besides, we investigated another refolding, Ki67 FHA domain by cryo-stopped-flow CD and SAXS. This protein also showed  $\alpha$ -helical burst and it is thought that the intermediate was also compact.

The present study demonstrates that folding landscape of these  $\beta$  proteins, including FHA domains, have at least main barrier(s). It is taken into consideration that the barrier(s) is (are) mainly by conformation entropy driven.