α-Helical burst on the folding pathway of FHA domains from Rad53 and Ki67

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

We have a simple question: do other β proteins also form α-helical intermediates on their folding pathway? So, we have studied other β 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°C. The phase I is the burst phase with the increase of α-helix. The phase II is observable (0.25 s⁻¹) phase, in which α-helix decreased. The phase III is slower than 30 min. When temperature increased to -15°C, the phase II became too fast to be detected. Actually, the CD value of the burst phase of -15°C is similar to the CD value of the last level of the phase II of -20°C. Insteads, another phase (α-helix decrease) appeared at -15°C. Judging from its CD value, it seems that the observable phase of -15°C corresponds to the phase III of -20°C. We also measured time-resolved SAXS at -20°C. From the Rg values, it is thought that α-helical burst intermediates at -15°C and -20°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 α-helical burst and it is thought that the intermediate was also compact.

The present study demonstrates that folding landscape of these β 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.