

## Kinetic and Equilibrium Intermediates Appeared in Src SH3 Domain

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An alpha-helix-rich intermediate (burst phase) was found and studied on the folding pathway of a beta-structure protein src SH3 domain. The alpha-helical content of the kinetic intermediate or transient intermediate (TI) was c.a. - 9,000 (deg.cm<sup>2</sup>.dmol<sup>-1</sup>) (CD value) and radius of gyration (Rg) of the TI was 18.5 Å. Judging from Kratky plot, the TI is compact.

The alpha-helix-rich bursts were found in many proteins. The alpha-helical contents of the burst phases are found to be proportional to the helical fraction predicted by Helix2 program with the correlation coefficient of 0.83 [1].

Meanwhile, a single mutant at 45 position of src SH3 domain from Ala → Gly took a helical rich intermediate at pH 3. This mutant was written in A45G. We called it equilibrium intermediate (EI) [2]. The ellipticity of EI at 222 nm was c.a. - 10,000 (deg.cm<sup>2</sup>.dmol<sup>-1</sup>), while Rg was estimated to be 19.1 Å. As far as CD and X-ray scattering data are concerned, it seems that TI and EI resemble with each other. The following figures showed the data of wild-type (WT) src SH3 domain and A45G at pH 3, which good explained the TI and EI.

### Conclusion

1.  $\alpha$ -helical burst (TI) appeared on the folding pathway of b-structured protein src SH3 domain.
2. The amplitude of  $\alpha$ -helical burst and the predicted  $\alpha$ -helical fraction by Helix2 are well correlated, which suggests the  $\alpha$ -helical burst was formed by collapse of  $\alpha$ -helices transiently formed.
3. A mutant, A45G showed an equilibrium intermediate (EI) at pH 3.
4. As far as Rg and CD are concerned, TI and EI resemble to each other.

### References

- [1] Li *et al.* (2007) *Biochemistry*, 46, 5072-5082.
- [2] Li *et al.* (2007) *J.Mol. Biol.*, 372, 747-755.