

α -helix-rich-states of β -lactoglobulin and src SH3, formed in high concentration of ethylene glycol and trifluoroethanol, are not either fully unfolded or compact

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Abstract Bovine β -lactoglobulin (BLG), quine β -lactoglobulin (ELG) are proteins which take predominant β -structure in the native state. We found that both proteins form α -helical inter mediate^{1,2)} at high concentration (80-90%) of ethylene glycol (EGOH). We performed X-ray solution scattering and found compact structure were lost at such high concentration of EGOH. This α -helix rich inter mediates are unfolded. We have found that both BLG and ELG form compact α -helix-rich intermediates on their refolding pathways, and ELG forms compact α -helix-rich equilibrium intermediate in acidic condition ^{1,2)}. The present results demonstrate that the intermediates appeared in high EGOH concentration is different from these compact intermediates.

We also investigate structural changes of BLG in trifluoro ethanol (TFE). TFE is well known to induce helical structure on proteins. α -helical content increased at more than 20% TFE in circular dichroism (CD) measurement. X-ray solution scattering could not detect clearly how structure changes. Strong scattering of TFE/water mixtures disturbed precise analysis.

We also investigate src SH3 domain, a small full β -sheeted protein of 57 residues. We performed TFE titration on CD measurement. CD spectrum indicates α -helical content increased at more than 15% TFE. From X-ray scattering, src SH3 is in compact state in 0 to 10% TFE. In 15% TFE, src SH3 lost compact structure but not fully unfolded. This structure is probably not same with the transient α -helix rich intermediate observed on refolding pathway³⁾.

- 1) Qin *et al.* (2001) FEBS lett. 507, 299-302.
- 2) Matsumura *et al.* (2008) Biophys. Chem. 134, 84-92
- 3) Li *et al.* (2007) Biochemistry, 46, 5072-5082