Structural analysis of α-helix rich intermediate of bovine β-lactoglobulin on its folding pathway

Zie-jie Qin1, Yoshitaka Matsumura2, Masaji Shinjo2 and Hiroshi Kihara2*

1 Department of Chemistry and Biochemistry, University of California at Santa Cruz, 95064, USA
2 Department of Physics, Kansai Medical University, Hirakata 573-1136, Japan

Introduction

We have reported that bovine β-lactoglobulin (BLG) took two intermediates (I1 and I2) on its early stage of folding at -28°C in the presence of 45% ethylene glycol1). Both I1 and I2 are rich in α-helix, though I2 is richer. To investigate structures of the intermediates, we have done cryo-stopped-flow monitored by X-ray solution scattering method. The shape of I2 was calculated by GASBOR program2).

Experiment

BLG forms dimmer at physiological condition and dissociate to monomer at acidic pH. At pH2, we performed time-resolved refolding experiments by circular dichroism (CD) and X-ray solution scattering methods. Experiments were done with stopped flow apparatus.

Result

Fig.1 shows θ222 in kinetic CD measurements1,3). α-helix rich intermediate (I1 and I2) are shown. The burst phase corresponds to I1. After increase of α-helix, θ222 keeps constant (I2) at this temperature. Fig.2 shows time-resolved radius of gyration (Rg). The first one point corresponds to the transient point from I1 to I2. But the difference of I1 and I2 is not clear. From X-ray scattering profile, we calculated molecular structure of I2 by GASBOR program.2 Fig.3 shows the calculated shape. Two domains structure was obtained.

The native structure of BLG looks to be composed of two divided structures as shown in Fig.4. It seems plausible that the two local structures were formed in the early stage of protein folding and combined into the native structure.

References

2) Svergun et al. (2001) Biophys. J. 80, 2946-2953
*kihara@makino.kmu.ac.jp

Fig.1 kinetic CD measurement of θ222 at -28°C1,3)

Fig.2 Rg obtained by kinetic SAXS measurement at -28°C. Broken line shows averaged Rg from 185ms to 2985ms.

Fig.3 Reconstructed structures of I2 (1085-1485ms) calculated by GASBOR.3) Large spheres express Ca atoms and small spheres express water molecules.

Fig.4 BLG crystal structure on PDB. (PDB ID: 1BEB :extract chain A) Red: 1-75 residues, Green: 74-85 residues, Blue: 86-160 residues