

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

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Introduction

We have reported that bovine β -lactoglobulin (BLG) took two intermediates (I_1 and I_2) on its early stage of folding at -28°C in the presence of 45% ethylene glycol¹⁾. Both I_1 and I_2 are rich in α -helix, though I_2 is richer. To investigate structures of the intermediates, we have done cryo-stopped-flow monitored by X-ray solution scattering method. The shape of I_2 was calculated by GASBOR program²⁾.

Experiment

BLG forms dimer 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 θ_{222} in kinetic CD measurements^{1,3)}. α -helix rich intermediate (I_1 and I_2) are shown. The burst phase corresponds to I_1 . After increase of α -helix, θ_{222} keeps constant (I_2) at this temperature. Fig. 2 shows time-resolved radius of gyration (R_g). The first one point corresponds to the transient point from I_1 to I_2 . But the difference of I_1 and I_2 is not clear. From X-ray scattering profile, we calculated molecular structure of I_2 by GASBOR program²⁾. 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

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 - 2) Svergun *et al.* (2001) Biophys. J, 80, 2946-2953
 - 3) Matsumura *et al.* (2008) Biophys. Chem. 134, 84-92
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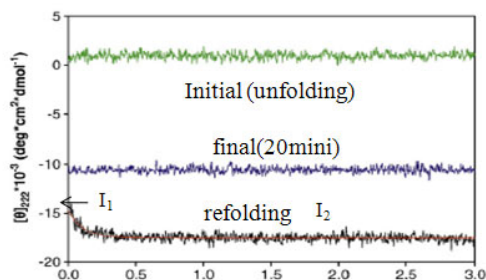


Fig.1 kinetic CD measurement of θ_{222} at -28°C ^{1,3)}

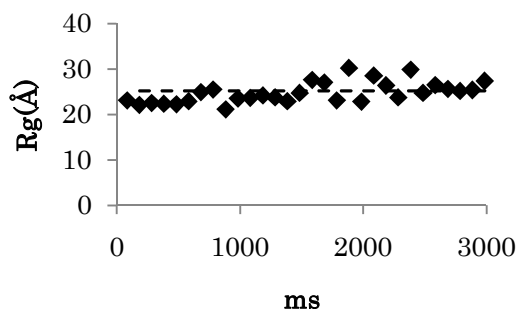


Fig.2 R_g obtained by kinetic SAXS measurement at -28°C . Broken line shows averaged R_g from 185ms to 2985ms.

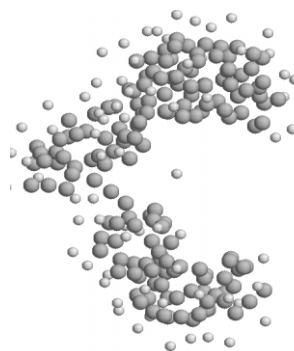


Fig.3 Reconstructed structures of I_2 (1085-1485ms) calculated by GASBOR²⁾. Large spheres express $\text{C}\alpha$ atoms and small spheres express water molecules.

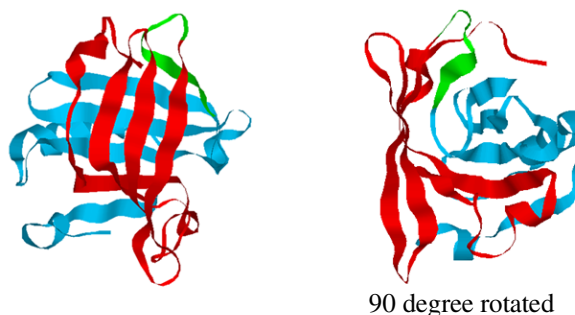


Fig.4 BLG crystal structure on PDB. (PDB ID: 1BEB :extract chain A) Red: 1-75 residues, Green: 74-85 residues, Blue: 86-160 residues