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α-helix-rich intermediates of BLG and ELG induced in 90% ethylene glycol

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Introduction

We have found that bovine β-lactoglobulin (BLG) and equine β-lactoglobulin (ELG) form α-helix rich intermediates at high concentration of ethylene glycol (EGOH) [1,2]. In the present study, solution structures of these proteins were investigated by x-ray solution scattering in the presence of various concentrations of EGOH.

Structures of BLG were also investigated in the presence of trifluoroethanol (TFE). TFE is known to induce α-helix on proteins. The interest is to compare α-helix-rich intermediates induced by EGOH and TFE.

Experimental

X-ray scattering experiments were done at the beamline of 15A, keeping the sample-to-detector-distance at c.a. 1.3 m,. with a CCD-based X-ray detector (Hamamatsu Photonics, C7300). The obtained data were corrected for distortion of images, non-uniformity of sensitivity, and the contrast reduction for and X-ray image intensifier. Temperature was controlled at 4±0.1 °C. Experiments were done at pH2 for BLG and at pH8.7 for ELG.

Results and Discussion

Fig. 1 shows EGOH concentration dependence of radius of gyration (Rg) for BLG and ELG. At 45% EGOH, Rgs are almost the same with the native states. At 90% EGOH, in contrast, both BLG and ELG show larger Rg.

Fig. 2 shows Kratky plots of BLG. At 0% and 45% EGOH, BLG was compact, showing peaks. At 90% EGOH, the plot shows gradual increase with no peaks, indicating BLG was unfolded. The same results were obtained for ELG. Judging these finding together with CD data [1, 2], both BLG and ELG form **unfolded α-helix- rich intermediates** in 90% EGOH.,

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 prestnt results demonstrate that the intermediates appeaed in high EGOH concentration is different from other intermediates so far reported [1, 2].

Fig. 3 shows the TFE concentration dependence of Rg of BLG. Between 5% and 20% TFE, Rg are bigger than the native state (0% TFE).

Kratky plots (Fig.4) indicate BLG takes compact structure between 0% and 15% TFE.

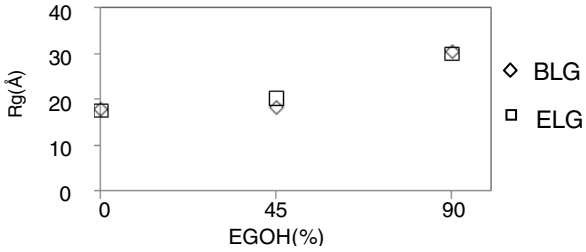

Fig.1 Rg of BLG and ELG against EGOH .

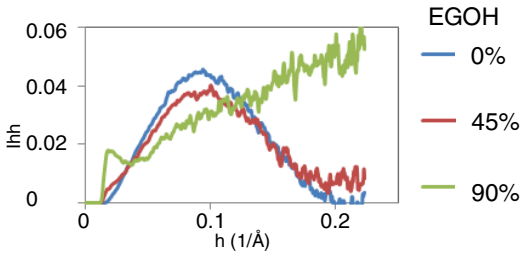

Fig.2 Kratky plot of BLG with EGOH

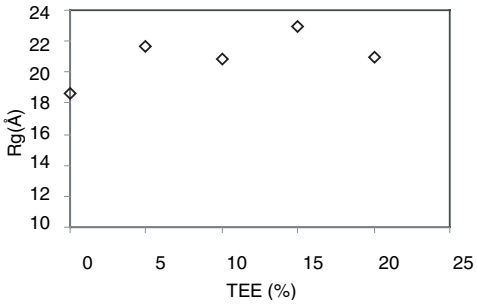

Fig.3 Rg of BLG against TFE.

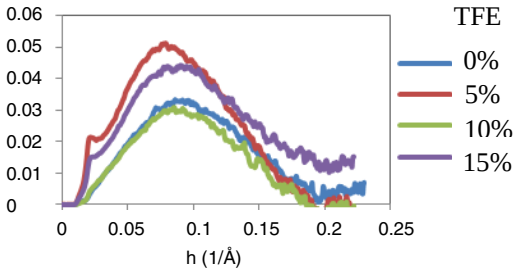

Fig.4 Kratky plot of BLG with TFE

References

[1] Qin *et al.* (2001) FEBS lett. 507, 299-302.
[2] Matsumura *et al.* (2008) Biophys. Chem. 134, 84-92.

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