10C/2006G394

Complex Formation of Sulfobetaine-type Surfactants with Proteins

Kenji Kubota*, Nobukazu Nameki and Kaori Wakamatsu
Gunma University, Kiryu, Gunma 376-8515, Japan

Introduction

β-Lactoglobulin (bovine, βLG) is a major component of milk and is a dimmer of 36.8 KDa in aqueous solution (pH~7). βLG molecule contains substantial β-strands, 51% β-strands and 7% α-helix. With decrease of pH (below pH 3), it dissociates into monomers. Addition of alcohol affects the dispersion state strongly, and results in aggregation at moderate alcohol concentration. It is also known that βLG forms stable intermediate state at proper solvent conditions.

Non-detergent sulfobetains (NDSB) are zwitterionic com-pounds, and are known to be effective for extraction, solubilization, renaturation and crystallization of proteins. In our earlier works NDSBs function to prevent the aggregation of proteins with binding to proteins mainly at β-strands region and with stabilizing the native confor-mation of protein molecules. Detailed analyses, e.g. identification of binding site and binding and stabilizing mechanism, are important in order to apply NDSBs for the structural studies of proteins.

Experimental

Bovine βLG was purchased from Sigma-Aldrich Co. and was used without further purification. NDSB-195 (MERCK Co.) was used as a typical NDSB (195 means molecular weight). Solvents are 20mM HCl and mixture of 20mM HCl and 20% isopropanol (iPA). Scattering functions (SAXS) of βLG without and with 0.5 M NDSB-195 were obtained at 25°C using BL-10C spectrometer. Wavelength of X-ray was 0.149 nm. Data analyses were carried out on the Guinier plot (radius of gyration), Kratky plot (globular conformation) and distance distribution function (P(r) function).

Results and Discussion

Guinier analyses showed clear dissociation of βLG dimmer into monomers, 2.03 nm (native state) in 20mM HEPES (pH 7.4) to 1.68 nm in 20mM HCl (monomeric state). Addition of iPA up to 20% in the solvent (20mM HCl) resulted in remarkable increases in radius gyration, 2.29 nm, indicating the unfolding of βLG. Further addition of NDSB-195 up to 0.5M caused a sharp decrease (1.88 nm). NDSB-195 works as a stabilizing agent and molten globule state was realized even in the unfolding solvent condition. These findings are verified in the following analyses of Kratky plots and P(r) functions.

Fig. 1 depicts the Kratky plots of βLG in the various solvents. In 20mM HCl / 20% iPA, $Q^2I(Q)$ does not show any peak and distinct plateau and monotonous increase at higher Q region was observed. The presence of plateau is characteristic of chain-like conformation. Such a feature is detected showing an extended tail and bimodal P(r) as shown in Fig. 2.

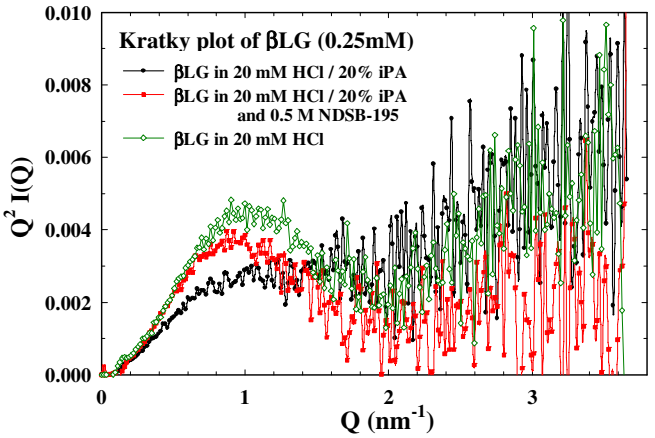

Figure 1. Kratky plots of βLG at 25°C. Concentration of βLG is 0.25 mM.

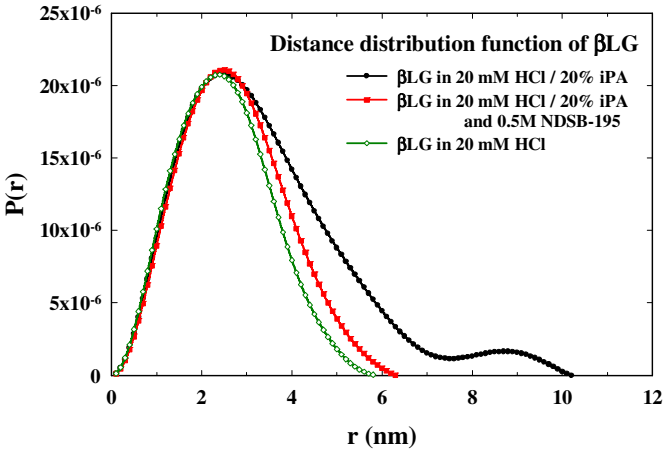

Figure 2. Distance distribution function of βLG.

Addition of NDSB caused a qualitative change in the molecular conformation. Clear peak appeared in the Kratky plot and bell-type unimodal P(r) was obtained. Size is a little larger than that in 20mM HCl. NDSB cancels the unfolding act by iPA and bLG remains in the molten globule state. Although the analyses of secondary structure of βLG in those states are necessary, NDSB should bind to β-strands portion and compact conformation was stabilized.

References

[1] Kogure et al. Trans. MRS-J.31, 787 (2006).
[2] Abascal et al. Biophys. J. 20, 1741 (2004).
[3] Bezancon et.al. Biophys. Chem 100, 469 (2003).