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The thermodynamic stability of the different forms of β-lactoglobulin (A and B) to sodium *n*-dodecyl sulphate

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Abstract

Bovine β-lactoglobulin (BLG) has two genetic variants known as BLG A and B. The interaction of sodium *n*-dodecyl sulphate (SDS) with its two variants has been studied by different experimental techniques such as titration microcalorimetry, equilibrium dialysis, UV spectrophotometry, and temperature scanning spectroscopy in acetate buffer at pH 3.2 and 25°C. The binding data have also been interpreted in terms of structural points using the Wyman theoretical model. The results from different techniques show that BLG B has greater thermodynamic stability than BLG A.

Keywords: β-Lactoglobulins (A and B); Sodium *n*-dodecyl sulphate (SDS); Titration microcalorimetry; Equilibrium dialysis

1. Introduction

Various studies of the interaction of globular proteins with anionic surfactants such as sodium *n*-dodecyl sulphate (SDS) have been carried out [1–3]. These interactions frequently lead to denaturation of the tertiary structure of proteins and formation of protein–surfactant complexes, so the study of the nature of the interactions between proteins and anionic surfactants can help us obtain information about the thermodynamic stability of proteins and the forces which are responsible for this stability.

β-Lactoglobulin (BLG) is a very abundant protein found in the milk of mammals [4, 5]. BLG has been studied extensively [6,7] and it is known that small non-polar ligands such as retinol, protoporphyrin IX and free fatty acids can bind to it [8–10].

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Under physiological conditions BLG consists of two non-covalently linked subunits. The monomer of BLG has a molecular weight of 18 400 corresponding to a chain of 162 amino acid residues [11]. At low temperature (below 25°C) the protein undergoes reversible self-association to a tetramer within the pH range 3.5–5.5 [12–16] which is a consequence of the very high hydrophobicity of the protein [17]. However BLG is monomeric in form at pH values below 3.5 [18]. There are two genetic variants, commonly known as BLG A and BLG B [19]. The tertiary structures of these proteins have been determined with 2.5 Å resolution, and there are some reports on the interactions of such proteins with SDS [20–23]. However, there are no studies comparing the interactions between these variants, which only differ by two amino acid residues [24], with SDS. This study is an attempt to compare the thermodynamic stability of the interactions of the two variants (BLG A and BLG B) with SDS using various physico-chemical experimental techniques.

2. Experimental

2.1. Materials

BLG A and B from bovine milk were purchased from Sigma Chemical Co as a three-times crystallized and lyophilized powder which was used without further purification. Sodium *n*-dodecyl sulphate (especially pure grade) was also obtained from Sigma. Visking membrane dialysis tubing (molecular weight cut-off 10 000–14 0000) was from SIC (Eastleigh, Hampshire, UK). Rosaniline hydrochloride dye was used as supplied by BDH. The acetate buffer, pH 3.2, $I = 0.05$, was prepared in double-distilled water.

2.2. Methods

2.2.1. Titration microcalorimetry

Enthalpy measurements were performed at 25.0 ± 0.005°C using an LKB microcalorimeter (2277 Thermal Activity Monitor, Boromma, Sweden). The microcalorimeter was interfaced with an IBM PS/2 Model 40 486 computer, thermometric Digitam 3 was the software program used. A 1000 μL injection syringe was employed throughout. The enthalpy of interaction between SDS and BLG was measured by transferring the SDC solution to the syringe and the BLG solution to a 5 ml titration cell. The concentration of SDS inside the syringe was 40 mM and the concentration of BLG in the titration cell was 0.2% *w/v*. The volume of SDS solution injected in each step was 25 μL. The enthalpy of demicellization of SDS due to injection was corrected by measuring the enthalpy change of injections of SDS solution into buffer solution using the identical procedures and experimental conditions. The heat of dilution of BLG is negligible.

2.2.2. Equilibrium dialysis

Equilibrium dialysis was carried out to determine the concentration of free SDS in equilibrium with the complexes and hence the amount of SDS bound to the protein.

The experiments were carried out with dialysis bags made from visking tubing (9/16 inch) as a semipermeable membrane. Free SDS concentrations were assayed by the rosaniline hydrochloride dye method [25]. Volumes of 2 mL aliquots of BLG solution at a concentration of 0.06% (*w/v*) were dialysed against 2 mL aliquots of different concentrations of SDS solution. Equilibrium times were over 96 h.

2.2.3. Temperature-scanning spectrophotometry

Absorbance profiles, which describe the thermal denaturation of BLG A and B, were obtained from a Gilford model 2400-S spectrophotometer fitted with a temperature programmer which controls the speed of temperature change in melting experiments. The cuvette holder can accommodate four samples; one acts as a reference buffer solution and the others for each experimental determination. The recording chart reads the temperature, reference line (from the reference cuvette) and the absorbance change at 278 nm for each of the three sample cuvettes.

2.2.4. Ultraviolet spectrophotometry

Measurements were made on a Shimadzu model 3100 double-beam spectrophotometer using matched 1 cm pathlength quartz cuvettes. The temperature of the cuvettes was maintained at 25°C using a separate water bath (model TB-85 Thermo Bath, Shimadu). The instrument reading was adjusted to zero with buffer solution in both cuvettes and the spectra subsequently obtained by replacing the buffer from the sample cuvette with the protein solution and adding identical portions of SDS solution to both cuvettes. The surfactant concentrations were in the range of 0–2 mM; the final proetin concentration being 0.1% (*w/v*).

All the measurements reported refer to surfactant concentrations below the critical micelle concentration of SDS. Calculations were made assuming a molecular weight of 18400 for BLG [26].

3. Results and discussion

Fig. 1 shows the UV spectra, between 240 to 300 nm, of BLG A and B in different concentrations of SDS. Absorbance changes due to protein unfolding by SDS at wavelengths of between 250 and 280 nm for variant A are shown to be larger than for variant B.

It is known that peaks occurring in the spectra of proteins in the region of 292 to 294 nm belong to Trp and that peaks below 270 nm belong to Phe [27]. The effect of SDS as a denaturant on the environment of Phe is larger than for Tyr and is reflected by the greater exposure with respect to Tyr and Trp of phenyalanine residues from the interior upon unfolding for both variants. However, the reverse effect has been seen in the denaturation of BLG by urea [28]. This illustrates the difference in the mechanism of denaturation by urea and SDS.

Fig. 2 shows the dependence of the absorbance at 278 nm on the total concentration and the number of SDS molecules bound by the two variants. It shows that the rate of absorbance change of variant A is more than for variant B so the stability of variant B with respect to denaturation by SDS is more than A.

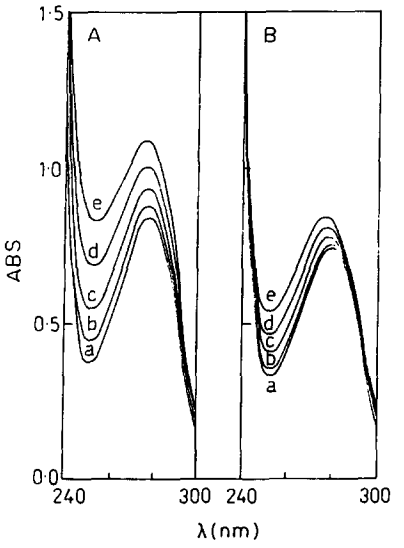

Fig. 1. UV spectra, 240 to 300 nm, of BLG A and B in acetate buffer at pH 3.2 and 25°C with different concentrations of SDS: (a) 0.0 mM, (b) 0.3 mM, (c) 0.6 mM, (d) 0.9 mM, (e) 1.2 mM.

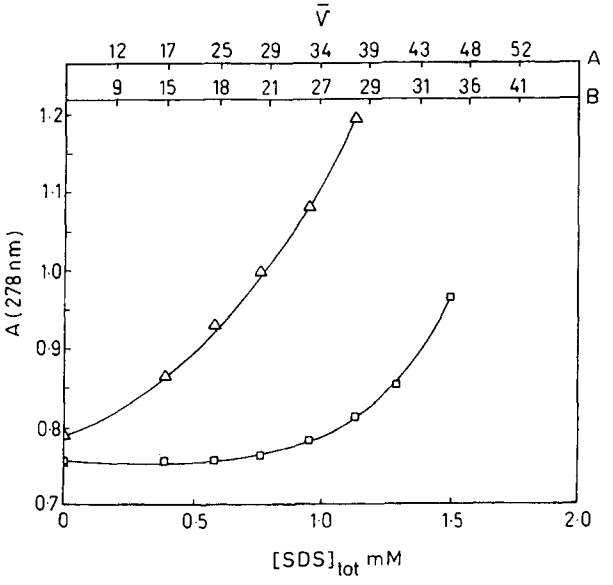

Fig. 2. Variation of absorbance at 278 nm with total concentration of SDS in acetate buffer at pH = 3.2 and 25°C. The upper axes show the number of moles of SDS bound per mole of BLG (obtained from binding experiments). (△) BLG A, (□) BLG B.

The binding isotherms plotted as the amount of bound SDS ($\bar{v}$) vs the log of free SDS concentration are shown in Fig. 3. All data results to free SDS concentrations and are below the critical micelle concentration of SDS which is 8.92 mmol L^{-1} at pH 3.2 [18].

The binding isotherms were fitted to an exponential equation as follows:

$$\bar{v} = a \exp \{b[SDS]_f\} \tag{1}$$

The average Gibbs free energy of binding per mole of SDS, $\Delta G_{\bar{v}_i}$, was calculated by application of the Wyman binding potential model [29]. The binding potential at any specified $\bar{v}_i \Pi_{\bar{v}_i}$ is calculated from the area under the binding isotherms according to the equation:

$$\prod_{\bar{v}_i} = 2.303\, RT \int_0^{\log [SDS]_{\bar{v}_i}} \bar{v}\, d \log [SDS]_f \tag{2}$$

where R and T are the universal gas constant and temperature in Kelvin, respectively. It is related to an apparent binding constant $K_{app}^{\bar{v}_i}$ as follows:

$$\prod_{\bar{v}_i} = RT \ln (1 + K_{app}^{\bar{v}_i} [SDS]_f^{\bar{v}_i} \tag{3}$$

Values of $K_{app}^{\bar{v}_i}$ were determined by application of Eqs. (2) and (3) to determine values of $\Delta G_{\bar{v}_i}$

$$\Delta G_{\bar{v}_i} = -\frac{RT}{\bar{v}_i} \ln K_{app}^{\bar{v}_i} \tag{4}$$

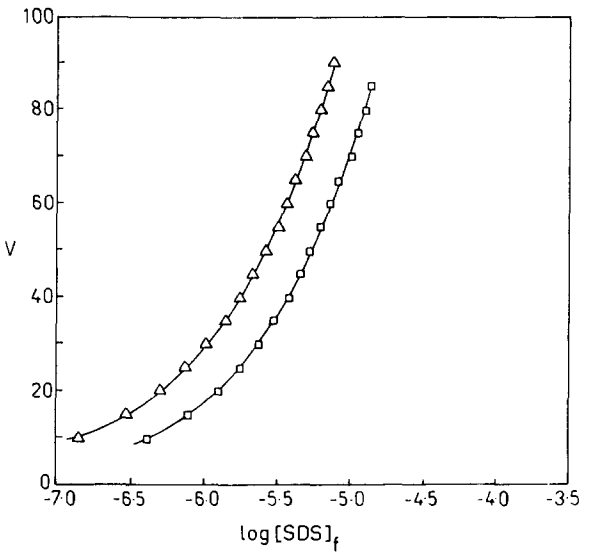

Fig. 3. Binding isotherms of BLG A (△) and BLG B (□) on interaction with SDS in acetate buffer pH = 3.2 and 25°C.

Fig. 4 shows $\Delta G_{\bar{v}_i}$ as a function of $\bar{v}_i$ at pH 3.2 for BLG A and B. The value of the free energy change, 2 kJ mol^{-1}, and the approximate number, 8–12, of bound SDS molecules ($\bar{v}$) is higher for variant A than for B in the complexation of SDS to BLG. This means more interaction occurrs for BLG A than for B, therefore variant B is more stable than variant A.

Fig. 5 shows the thermal profiles for the two BLG variants obtained using a Gilford spectrophotometer. The melting temperatures of variants A and B, which are obtained from the midpoints of their sigmoidal thermal denaturation curves, are 80.5 and 82°C respectively.

The essential feature of this study is the observed similarity between the effects of SDS and temperature on the process of denaturation. However, the change of absorbance with respect to the concentration of SDS for BLG A is more than it is for BLG B.

Fig. 6 shows the enthalpies of calorimetry per bound SDS molecule ($\Delta H_{\bar{v}_i} = \Delta H_{cal}/\bar{v}$) vs $\bar{v}_i$ the final concentration of SDS.

The variation of $\Delta H_{\bar{v}_i}$ shows that the interaction of SDS and BLG B is more exothermic than for A. The two enthalpy curves in Fig. 5 are similar in shape, both exhibit maximum and minimum features. By interpretation, the measured calorimetric enthalpy consists of at least two contributions; the enthalpy of binding, which is usually exothermic, and the enthalpy of unfolding, which is often endothermic. Thus the shape of this plot indicates protein unfolding, which is endothermic process, within the exothermic binding of SDS. This endothermic process, however, occurred at a lower

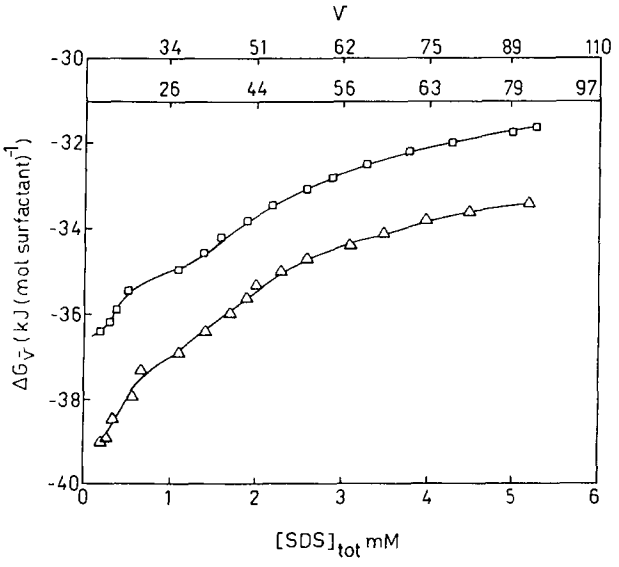

Fig. 4. Variation of $\Delta G_{\bar{v}_i}$ with total concentration of SDS for binding of SDS to BLG in acetate buffer at pH = 3.2 and 25°C. The upper axes show the number of moles of SDS bound per mole of BLG, (△) BLG A, (□) BLG B.

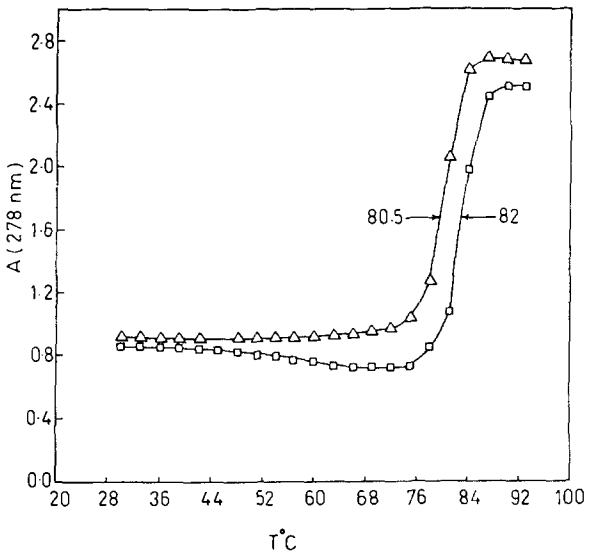

Fig. 5. Variation of absorbance of BLG at 278 nm in acetate buffer at pH = 3.2 with temperature (°C) measured using a Gilford spectrophotometer. (△) BLG A, (□) BLG B.

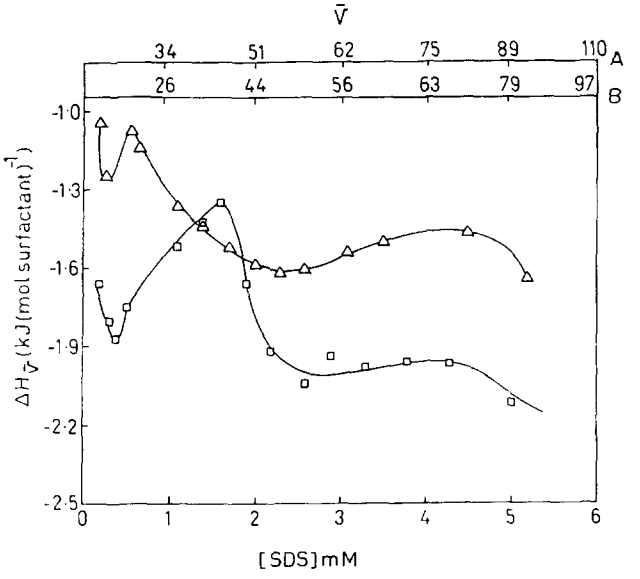

Fig. 6. The variation of enthalpy per mole of surfactant, $\Delta H_{\bar{V}}$, with total concentration of surfactant for the interaction of BLG with SDS in acetate buffer at pH = 3.2 and 25°C. The upper axes show the number of moles of SDS bound per mole of BLG.

total concentration and $\bar{v}$ of SDS for BLG A than for BLG B; this is in agreement with the other results and shows the stability for BLG A to be less than B.

The height of the maxima in the two enthalpy curves can be related to the amount of unfolding enthalpy. Accordingly, it can be said that the unfolding enthalpy (heat content) of variant B is more than that of A.

The greater thermodynamic stability of BLG B with respect to A can be interpreted by considering the difference in the primary structures of these two variants. These difference occur at positions 64 and 118 where Asp and Val in BLG A are replaced, respectively, by Gly and Ala in BLG B [24]. There is region with negative charge density (Glu(62), Asn(63), Asp(64) and Glu(65) in BLG A which repels the end carboxyl group and destabilizes the tertiary structure [24]. The replacement of Asp(64) by Gly residue in BLG B means that this repulsion is reduced and the tertiary structure becomes more stable.

There are data in the literature which substantiate our result e.g. the greater resistance of BLG B than BLG A to the proteolytic effects of proteases [30] and the greater reactivity of the –SH group in BLG A in reaction with 5,5′-dithiobis 2-nitrobenzoic acid (DTAB) and 2,2′-dithio dipyridine (2-DTP) [31].

In conclusion, results for the interaction of BLG (A, B) with SDS obtained by various physico-chemical techniques (e.g., microcalorimetry, equilibrium dialysis, temperature scanning spectroscopy and UV spectrophotometry), indicate that the structure of BLG B is markedly more stable than that of BLG A.

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