The State of Aggregation of Red Deer (*Cervus elaphus* L.) β -Lactoglobulin Preparations near Neutral pH

By E. IAN McDougall and JAMES C. STEWART
Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, Scotland, U.K.

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1. The state of aggregation of four red-deer (*Cervus elaphus* L.) β -lactoglobulin preparations and a control ox β -lactoglobulin A preparation was studied by sedimentation-equilibrium experiments at pH 6.5 and 20°C. 2. Three of the deer preparations and the ox control each behaved as a monomer-dimer system, with a value of $\log K$ (where K is the association constant in litres/mol) in the range 5.4–5.5. 3. When one of these deer preparations was examined in the presence of dithiothreitol, $\log K$ appeared to decrease to 4.5. 4. One deer preparation, comprising recovered material, appeared to have undergone irreversible changes and to behave like a non-equilibrating system containing monomer, dimer and trimer. 5. The sedimentation-equilibrium properties of the deer monomer was studied in 6M-guanidine hydrochloride at pH 7.0; the mol.wt. was 17600, the second virial coefficient was $3.4 \times 10^{-3} \text{ ml} \cdot \text{mol}^{-1} \cdot \text{g}^{-2}$, and the apparent partial specific volume 0.724 ml/g, a value indicating an appreciable decrease in volume on dissociation and denaturation.

The isolation of two red-deer (*Cervus elaphus* L.) homologues of ox (*Bos taurus*) β -lactoglobulins, and some properties of the more commonly occurring variant (pI 5.17), were described previously (McDougall & Stewart, 1976). Sedimentation experiments, gel chromatography and amino acid analysis indicated that, like the ox protein (see the review by McKenzie, 1971), it existed in dilute buffer near neutral pH and 20°C mainly as a dimer of an 18000-mol.wt. monomer, and appeared to swell when the pH was raised to 8.2, and aggregated irreversibly at a more alkaline pH and low temperature. Some sedimentation-equilibrium experiments were reported briefly in that paper (McDougall & Stewart, 1976). To extend the comparison, the results of these and additional sedimentation-equilibrium experiments, all near neutral pH and 20°C, are presented here more fully. The state of aggregation of different preparations in dilute buffer is examined in relation to its stoichiometry and possible association equilibrium and compared with that of ox β -lactoglobulin under the same conditions (to provide an equilibrating monomer-dimer control). Some physical properties of the monomer were determined from experiments in the dissociating solvent 6M-guanidine hydrochloride.

Materials and Methods

The materials used to isolate the β -lactoglobulin fraction from pooled deer-milk samples were described by McDougall & Stewart (1976). Their use in the different preparations is indicated briefly in Table 1. The material was eluted from the columns

as a resolved or as the only component. Further, gel chromatography of preparations ox A and deer 2, 4 and 4A in columns (100 cm $\times$ 5 cm or 150 cm $\times$ 0.9 cm) of Sephadex G-75 showed only one component. Starch-gel electrophoresis of preparations ox A and deer 2, 4, 4A and 5 showed only one zone after 11 cm migration. This was taken to indicate a suitable preparation. However, in a study of the isoelectric point, isoelectrofocusing experiments showed that preparation 2 contained a small amount of the less common variant. In the subsequent preparations 4 and 4A, a step was added to remove it. The amount does not seem to have been significant, as the results of the sedimentation-equilibrium experiments on these preparations could be combined (see below). The purity of preparation 4 was supported by the many almost integral numbers of residues found in the amino acid analysis and the rapid increase in the u.v. absorbance below 300 nm; dilutions of the preparation in dilute buffer and in 6M-guanidine hydrochloride, at pH 6.5, with an $A_{278}^{1\%}$ of 0.9, showed an A_{315} of 0.01 and an A_{350} of 0.005. Preparation 5 was obtained from pooled milk samples from which the source of the less-common variant had been excluded. Gel chromatography on a column (90 cm $\times$ 1 cm) of Sepharose 6B in 6M-guanidine hydrochloride showed that it was eluted almost entirely as the monomer, with some hardly discernible material being eluted at about the dimer position. Preparation 2A comprised samples of preparation 2 that had been diluted for various determinations, after which the remains were combined, concentrated and dialysed.

Table 1. *Summary of sedimentation-equilibrium experiments on β -lactoglobulin preparations at pH 6.5 and 20°C*
 The apparent cell weight ($\bar{M}_w$)- and Z-average ($\bar{M}_z$) molecular weights, $\pm$ S.E., are given, with the numbers of determinations in parentheses. The composition of the buffer, initial concentrations (range 0.04–0.37 g/ml) and speeds (range 20000–26000 rev./min) are given in the legend to Fig. 1. Equilibrium patterns were evaluated usually after 12 h and 24 h centrifugation. The last column refers to the appropriate part of Fig. 1, in which the distributions of the point weight-average molecular weights in the individual experiments are shown.

Preparation	Isolation procedure	$10^{-3} \times \bar{M}_w$	$10^{-3} \times \bar{M}_z$	Reference to Fig. 1
Ox A	Gel chromatography of commercial preparation	33.9 ± 0.8	35.6 ± 0.9 (6)*	(a)
Deer 2	Gel chromatography	33.3 ± 0.2	35.0 ± 0.6 (14)	(b), (c)
4	Gel chromatography and electrofocusing			
4A	Gel and ion-exchange chromatography			
5	(NH ₄) ₂ SO ₄ precipitation and ion-exchange chromatography			
5†	(NH ₄) ₂ SO ₄ precipitation and ion-exchange chromatography	28.0 ± 0.6	31.8 ± 1.2 (4)	(d)
2A	Gel chromatography and recovery (see the text)	29.3 ± 1.6	35.6 ± 2.5 (14)	(e)

* Data from Table 2 in McDougall & Stewart (1976).

† Experiments in buffer containing dithiothreitol.

The equilibrium experiments used the high-speed low-concentration method of Yphantis (1964), in the long-column modification described by Chervenka (1970). In some experiments, a more extended usable distribution of concentration in the column was obtained by some lowering of speed, as discussed by Teller *et al.* (1969). Sample (0.05 ml) was layered in a 12 mm capillary synthetic-boundary cell under the solvent against which it had been dialysed to give a 7 mm column. The dialysis time was overnight for the dilute buffer and 4 days for 6M-guanidine hydrochloride. The former was buffered at pH 6.5, and in one series of experiments contained dithiothreitol as a thiol-group protector; the guanidine hydrochloride solution was buffered at pH 7.0 and contained both EDTA and dithiothreitol to prevent cross-links in the denatured protein (for details see the legends to Figs. 1 and 3). No oil base was used in the sample and solvent columns, except in the few experiments where this is indicated; the oil used in these experiments was the fluorocarbon FC 43, obtained from Beckman-RIIC, Glenrothes, Fife, Scotland, U.K.

Each experiment was evaluated from interference photographs at 546 nm, for at least two different times of centrifugation, to ensure that equilibrium had been reached. For the evaluation, low-speed re-mix baselines were used, as described by Horbett & Teller (1972). The datum for the concentration scale, a depleted region near the air/buffer meniscus, was checked by a plot of the net fringe displacement against radial position in this region. In a few experiments it was uncertain whether this region was depleted, and an approximate value for the concentration there was obtained by a graphical method based on that of Teller (1973a). The correction was usually only a few micrometres, and a better estimate was not sought, except in two experiments

for which a bigger correction was required (25 and 20 μ m). This was obtained by using the 'two-species plot' described below.

The number- and weight-average molecular weights at different radial positions in the cell (the point values) and the weight- and z-average molecular weights averaged over the cell contents (the cell values) were calculated as described by Yphantis (1964) by using a FORTRAN program on an IBM 1130 computer. The cell number-average molecular weight was calculated as described by Lansing & Kraemer (1935).

The concentration in g/dl is required in giving the initial concentration used and for the calculation of equilibrium constants and virial coefficients. It was obtained from the interference measurements by using a value for the mean fringe separation of 284 μ m and 1 g/dl = 39.9 fringes in dilute buffer. The latter value was calculated for 546 nm from the specific refractive increment at 579 nm at 20°C, with adjustment for dispersion as described by Perlmann & Longworth (1948). For experiments in 6M-guanidine hydrochloride, a value of 1 g/dl $\equiv$ 27.9 fringes was used. This was based on a value of 0.7 for the ratio of the refractive increments in the two solvents, taken from the measurements of Munk & Cox (1972) on serum albumin and ovalbumin. For experiments on ox β -lactoglobulin, a value of 1 g/dl $\equiv$ 40.2 fringes in dilute buffers was taken from Albright & Williams (1968). The initial concentration was obtained from the fringe count across the boundary formed at the start of the equilibrium experiment. At equilibrium the concentrations at different radial positions were in the range 0–0.35 g/dl.

Further details of the preparations, materials, ultracentrifugation and ancillary measurements, gel chromatography, dry-weight determination and other

matters referred to here were given previously (McDougall & Stewart, 1976).

Results and Discussion

In evaluating the experiments, some simplifications were made. In the experiments in dilute buffer, the second virial coefficient was neglected in calculating molecular weights, since the protein was taken to be globular and all concentrations were within the range given by Teller (1973*b*) for the ideal behaviour of globular proteins. The protein concentrations were also in the range covered by the experiments of Zimmerman *et al.* (1970) on ox β -lactoglobulin at pH 6.9 and I 0.13, in which they found that a zero virial coefficient was suitable. Density gradients caused by solute redistribution were taken to be insignificant at the low concentrations used (Yphantis, 1964); any gradients arising from the redistribution of the third component, guanidine hydrochloride, in experiments in 6M-guanidine hydrochloride were also ignored, since Munk & Cox (1972) showed their effect to be very small. In calculating association constants, the partial specific volumes of monomer and dimer were assumed to be the same; changes in electrostatic free energy and pressure effects were not considered.

Experiments in dilute buffer

The equilibrium experiments were carried out at various initial concentrations and speeds; these are given in the legend to Fig. 1. The cell weight- and Z -average molecular weights were used to group the preparations according to differences in their behaviour. This is shown by the average values for these molecular weights given in Table 1. Comparison of the cell weight- and Z -average molecular weights suggested that the deer preparations 2, 4 and 5 had about the same degree of heterogeneity as the ox β -lactoglobulin preparation and that preparation 2A was the most heterogeneous one. The experiments on preparation 5 showed that, in the presence of dithiothreitol, lowered cell average molecular weights were obtained.

The nature of these differences appeared more clearly when the distribution of the point average molecular weights was considered. The distributions of the point weight-average molecular weight as a function of the concentration at different radial positions in the cell, for experiments with different initial concentrations, are shown in Figs. 1(a)–1(e). The repeatability of the distributions is illustrated by the duplicate experiments at three different initial concentrations included in Fig. 1(e).

The stoichiometry of the association indicated by the distributions was examined by the 'two-species plot' of Roark & Yphantis (1969), by using the point

number- and weight-average mol.wts. and the monomer wt. value of 18000 (see Fig. 2 legend). This plot also indicates any departure from ideal behaviour and erroneous concentration values at the air/buffer meniscus. The half-open circles (◐) in Fig. 2(b) show the effect of an underestimate of this concentration (see also Teller, 1973*c*). A corrected value was obtained by repeating the calculations for successive increments in the concentration until the curvature towards the origin was eliminated.

An association constant was calculated for those preparations for which the distributions of the point weight-average molecular weights in experiments using different initial concentrations appeared to be fairly compatible. The data were combined to give a single graphical evaluation of the integral of Steiner (1952) and values for the weight fraction monomer ($x_{1,r}$) as a function of the total concentration (c_r) at a given radial position (r). The plot of $(1 - x_{1,r})/x_{1,r}^2$ against c_r (up to $c_r = 2-3$ mm) gave a straight line passing near the origin ($c_r = 0-50 \mu\text{m}$), indicative of an ideal monomer-dimer system in equilibrium. From this the value of $\log K$ was obtained, where K is the association constant in litres/mol. The value was used to calculate the association curves included in Figs. 1(a)–1(d).

The distributions of the point weight-average molecular weights of the deer preparations 2, 4 and 5 (see Figs. 1*b* and 1*c*) were rather similar to that of the bovine β -lactoglobulin A shown in Fig. 1(a). Fig. 1(b) also shows that, when the lower of the two concentrations of preparation 4 was examined at two speeds, the experiment at the higher speed (Δ) yielded somewhat lower values, suggesting that some non-equilibrating component was present. The 'two-species plots' for these deer preparations followed the monomer-dimer locus, as shown in Figs. 2(a) and 2(b) (◐). This was also true for the experiments on the bovine protein (results not shown). The association constants obtained for the deer and ox preparations were in the range giving $\log K = 5.4-5.5$ at pH 6.5 and 20°C.

For the above values of $\log K$ it can be calculated that the concentration of protein containing equal amounts of monomer and dimer would give a net fringe displacement of about $150 \mu\text{m}$. As the calculations of the point average molecular weights become unreliable at fringe displacements approaching $100 \mu\text{m}$, the usable data refer to solutions containing a high proportion of dimer. This is suitable for determining the stoichiometry, but the strong association makes for a less-sensitive measurement of the association constant, a point discussed by Teller *et al.* (1969). In making comparisons with previously reported values for the association constant of ox β -lactoglobulin, it suffices to use the form $\log K$, transforming the results where needed. McKenzie & Sawyer (1972) obtained values of 4.2

Fig. 1. Weight-average molecular weights (M_w), as a function of concentration at different radial positions in the cell, for β -lactoglobulin preparations at pH 6.5 and 20°C, from experiments at different initial concentrations

Alternate points have usually been plotted to avoid overcrowding the diagram. No oil base was used except where indicated. The continuous lines are the association curves calculated by using a monomer mol.wt. of 18364 for ox and 18000 for deer β -lactoglobulin. The buffer used was 0.15M-NaCl/0.05M-imidazole/HCl, pH 6.5, I 0.19. (a) Ox β -lactoglobulin A: ●, initial concn. 0.20 g/dl, 22 h at 21740 rev./min; ▲, 0.10 g/dl, 29 h at 24630 rev./min; ■, 0.05 g/dl, 24 h at 25980 rev./min. The continuous line is for $\log K = 5.4$. (b) Deer preparation 2: ●, initial concn. 0.11 g/dl, 23 h at 25980 rev./min, with oil base. Deer preparations 4 and 4A: ○, initial concn. 0.11 g/dl, 29 h at 24630 rev./min; ▲, 0.04 g/dl, 24 h at 25980 rev./min; △, 0.04 g/dl, 25 h at 29500 rev./min. The continuous line is for $\log K = 5.4$. (c) Deer preparation 5: ●, initial concn. 0.37 g/dl, 17 h at 20410 rev./min; ▲, 0.21 g/dl, 21 h at 20410 rev./min; ■, 0.08 g/dl, 24 h at 20410 rev./min; ○, initial concn. 0.36 g/dl, 24 h at 25980 rev./min. The continuous line is for $\log K = 5.5$. (d) Deer preparation 5 in buffer containing 0.05M-dithiothreitol: ●, initial concn. 0.31 g/dl, 24 h at 20410 rev./min; ▲, 0.22 g/dl, 26 h at 20410 rev./min. The continuous line is for $\log K = 4.7$. (e) Deer preparation 2A: ●, initial concn. 0.33 g/dl, 27 h at 24630 rev./min with oil base (single experiment). ●, ○, initial concn. 0.25 g/dl; ▲, △, 0.10 g/dl; ■, □, 0.04 g/dl; (duplicate experiments), all for 24 h at 25980 rev./min. These experiments could not be combined to give a single value of $\log K$ (see below).

and 5.1 for the A and B variants at pH 7.5 and 20°C; Zimmerman *et al.* (1970) found values of 4.7 and 5.2 respectively at pH 6.9 and 20°C. In each case the sedimentation equilibrium method was used. Georges *et al.* (1962), using the light-scattering method, obtained results showing that the B variant had a value of 5.8 at pH 6.5 and 20°C. The present results

appear to be in reasonable agreement with these values, considering the change in value with pH.

The effect of adding dithiothreitol to the buffer in the experiments on preparation 5 (cf. Figs. 1c and 1d) was to make the point weight-average molecular weight start from lower values and increase more slowly with concentration. The 'two-species plot'

Fig. 2. 'Two-species plot' for red-deer β -lactoglobulin preparations

$M_{w,r}$ and $M_{n,r}$ are the weight- and number-average molecular weights at different radial positions in the cell; M_1 is the monomer mol.wt. A value for the latter of 18000, obtained by gel chromatography in 6M-guanidine hydrochloride and the amino acid analyses (McDougall & Stewart, 1976), was used. (a) Preparations 2 and 4. (b) Preparation 5: ○, experiments in buffer alone; ●, ▲, experiments in buffer containing dithiothreitol. For details of all experiments, see the preparation number and symbol in the legend to Fig. 1. The experiments on preparation 5 in buffer alone at the lower speed gave points that were too congested near the region of the upper convergence of the monomer-dimer locus and the hyperbola of homogeneity to be shown here. The points shown as half-open circles (◐) were obtained from the same experiment on preparation 5 in the presence of dithiothreitol as the filled circles; they indicate that an erroneous value for the concentration at the meniscus had been used (see above).

(see Fig. 2b, ●, ▲) still followed the monomer-dimer locus. The two experiments shown in Fig. 1(d) appeared to be consistent in indicating a less strong association. The extrapolations for the first term of the Steiner integral was more uncertain than in the other experiments. A value of $\log K = 4.5$ was obtained. Different values (4.4, 4.7) would have fitted the experimental points better at the lowest and highest concentrations.

The experiments on preparation 2A (Fig. 1e) showed a strong dependence of the point weight-average molecular weights on the initial concentration used. It was displaced to lower values in experiments with higher initial concentrations. A similar dependence on initial concentration was shown for a simulated system with heterogeneity of association constants by Roark & Yphantis (1969) and experimentally for a yeast aldolase preparation

by Harris *et al.* (1969). The present preparation behaved as if it contained some non-equilibrating or weakly associating form of monomer, and no association constant could be calculated. The 'two-species plot' (not shown) gave points which lay between the monomer-dimer and monomer-trimer loci, and this suggested that some trimer was also present.

Experiments in 6M-guanidine hydrochloride

For evaluating the sedimentation-equilibrium experiments in 6M-guanidine hydrochloride, our experimental value for the buoyancy factor ($\partial\rho/\partial c_2$) was based on measurements of density (ρ) on a series of dilutions (concentration = c_2) of a weighed amount of dried protein with 6M-guanidine hydrochloride. These gave the value, $\pm$ s.e., at constant molality of guanidine hydrochloride at 20°C, of 0.1645 ± 0.0004 (6). From the discussion below, this is probably not very different from the value at constant chemical potential of diffusible solutes; it was used to calculate the apparent molecular weights.

The plot of the reciprocal of the three apparent molecular-weight averages over the whole cell against the normalized concentration at the base of the cell at equilibrium is shown in Fig. 3 for experiments with different initial concentrations. The line of least-squares fit is included, from which the monomer molecular weight and second virial coefficient were obtained. The value for the monomer mol.wt., 17600, was slightly less than expected from the analytical gel chromatography and amino acid analysis of the protein, but does not justify changing the value of 18000 for the calculations in the 'two-species plot' and of the association constant of the protein in dilute buffer. The second virial coefficient, B , indicated a non-ideal solution, with $10^3 B = 3.4 \text{ ml} \cdot \text{mol} \cdot \text{g}^{-2}$. This is higher than the average value for ten proteins ($1.77 \text{ ml} \cdot \text{mol} \cdot \text{g}^{-2}$) quoted by Munk & Cox (1972); it is also higher than double the value ($1.08 \text{ ml} \cdot \text{mol} \cdot \text{g}^{-2}$) obtained for ox β -lactoglobulin by Lepanje & Tanford (1967) from osmotic-pressure measurements at 25°C, but it compares with the values of $3.36\text{--}4.30 \text{ ml} \cdot \text{mol} \cdot \text{g}^{-2}$ found by Harris *et al.* (1969) for yeast aldolase and $3.0 \text{ ml} \cdot \text{mol} \cdot \text{g}^{-2}$ found by Teller *et al.* (1969) for D-glyceraldehyde 3-phosphate dehydrogenase.

Some of our sedimentation-equilibrium experiments were carried out with an oil base in the sample and solvent columns; these experiments were examined for any untoward effects of the oil. In Fig. 1(b) the distribution of the point weight-average molecular weights in the experiment on preparation 2 with an oil base showed good agreement with the distribution in an experiment in preparation 4 for which no oil base was used. In Fig. 1(e), the distribution in the experiment on preparation 2A

Fig. 3. Reciprocal apparent cell molecular-weight averages as a function of the normalized concentration at the base of the cell, for red-deer β -lactoglobulin, in 6M-guanidine hydrochloride at pH7.0 and 20°C

$\bar{M}_a$ is the apparent cell average molecular weight. $\bullet$, $\circ$, Z-average, Δ , $\triangle$, weight-average, $\blacksquare$, $\square$, number-average molecular weights. Closed symbols, after 48 h centrifugation; open symbols, after 55 or 71 h centrifugation. The conditions were: initial concn. 0.36 g/dl and 33450 rev./min; 0.25 g/dl and 33450 rev./min; 0.14 g/dl and 35600 rev./min. The solvent was 6M-guanidine hydrochloride/0.1M- KH_2PO_4 /0.05M-dithiothreitol/0.01M-EDTA/KOH, pH7.0. The concentrations at the base of the cell were normalized for the different averages, as described by Harris *et al.* (1969), so that the results for a homogeneous preparation should form one line. The line of least-squares fit for 15 points is shown. From the intercept a value for M_1 of 17600 ± 300 ($\pm$ S.E.) is obtained. The slope gives $10^5 B = 3.4 \pm 0.3$ ($\pm$ S.E.) $\text{ml} \cdot \text{mol} \cdot \text{g}^{-2}$, where B is the second virial coefficient as defined by Williams *et al.* (1958).

with an oil base appeared consistent in relation to its initial concentration with the distributions in the other experiments on this preparation. Also, examination of the interface at the bottom of the column at equilibrium, in the experiments with an oil base, showed no discernible thickening, such as might be due to material accumulating there. The disadvantage of the use of oil was probably over-emphasized in our previous paper (McDougall & Stewart, 1976). Whereas it appears to be a precaution not to use oil, the effect of using it in the present experiments in dilute buffer at pH6.5 is thought to be small compared with that shown by Adams & Lewis (1968) for the bovine A variant at pH4.6 and a concentration of 2g/dl. This view is supported by the experience of McKenzie & Sawyer (1972); they found no difficulty when using an oil base in experiments on ox β -lactoglobulins at pH7.5.

The present results show that the similarities of

the deer to the ox β -lactoglobulin extend to the state of aggregation near neutral pH, where it exists as a strongly associating monomer-dimer system with an association constant close to that of ox β -lactoglobulin A. This association appeared to be perturbed by the presence of a thiol compound in the buffer. It is not clear how this could be brought about. Cecil & McPhee (1959) mention competition of thiol compounds with a thiol group as one explanation for their dissociating effect on certain proteins. Foster (1968) discusses a thiol-catalysed rearrangement of intrachain disulphide bonds as a possible source of microheterogeneity in ox plasma albumin. McKenzie *et al.* (1972) concluded that there were two arrangements in ox β -lactoglobulins. If this is true for the deer protein, the monomers might have different association properties. However, from the similarities to ox β -lactoglobulins, one would not expect the intrachain bonds to be very accessible, nor a thiol group to be very reactive, under the conditions used. It would be desirable to know whether the association of the ox β -lactoglobulin is similarly affected by thiol compounds.

The association of the deer β -lactoglobulin was studied at pH6.5 in order to be below the region of conformational change, which in ox β -lactoglobulin is about pH7.5 (Tanford *et al.*, 1959); the change is regarded as a preliminary to dissociation and irreversible aggregation at more alkaline pH values. However, this did not prevent preparation 2A, derived from a preparation originally obtained as a single component on gel chromatography, after dilution, storage and recovery, behaving as if in an early stage of irreversible aggregation. Presumably the small amount of dissociation is sufficient for these changes to take place slowly in this preparation and possibly to a lesser extent in others.

The buoyancy factor for deer β -lactoglobulin in 6M-guanidine hydrochloride provides some information on the volume changes accompanying the dissociation and denaturation of the protein. From this factor an apparent partial specific volume of 0.724 ml/g was calculated. This is less than the experimental value that we obtained for the partial specific volume in dilute buffer (0.748 ml/g). There do not appear to be any additional new volume changes due to solvent interactions, as the partial specific volume calculated from our amino acid analyses, by using the method of Lee & Timasheff (1974a) to allow for these interactions, was 0.749 ml/g. A similar decrease in volume not accounted for by solvent interactions was obtained for ox β -lactoglobulin by Lee & Timasheff (1974b). The apparent volume changes per 18000g were about 430 and 400 ml for the deer and ox proteins respectively. This appears to be a noteworthy feature of β -lactoglobulins.

From these results on the association and

denaturation of the deer β -lactoglobulin, it appears that the details of its tertiary and quaternary structure must be very similar to those of ox β -lactoglobulin.

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References

- Adams, E. T. & Lewis, M. S. (1968) *Biochemistry* **7**, 1044–1053
- Albright, D. A. & Williams, J. W. (1968) *Biochemistry* **7**, 67–78
- Cecil, R. & McPhee, J. R. (1959) *Adv. Protein Chem.* **14**, 255–389
- Chervenka, C. H. (1970) *Anal. Biochem.* **34**, 24–29
- Foster, J. F. (1968) *Chem. Soc. Spec. Publ.* no. 23, 39–41
- Georges, C., Guinand, S. & Tonnelat, J. (1962) *Biochim. Biophys. Acta* **59**, 737–739
- Harris, C. E., Kobes, R. D., Teller, D. C. & Rutter, W. J. (1969) *Biochemistry* **8**, 2442–2454
- Horbett, T. A. & Teller, D. C. (1972) *Anal. Biochem.* **45**, 86–99
- Lansing, W. D. & Kraemer, E. O. (1935) *J. Am. Chem. Soc.* **57**, 1369–1377
- Lee, J. C. & Timasheff, S. N. (1974a) *Arch. Biochem. Biophys.* **165**, 268–273
- Lee, J. C. & Timasheff, S. N. (1974b) *Biochemistry* **13**, 257–265
- Lepanje, S. & Tanford, C. (1967) *J. Am. Chem. Soc.* **89**, 5030–5033
- McDougall, E. I. & Stewart, J. C. (1976) *Biochem. J.* **153**, 647–655
- McKenzie, H. A. (1971) in *Milk Proteins* (McKenzie, H. A., ed.), vol. 2, pp. 258–330, Academic Press, London and New York
- McKenzie, H. A. & Sawyer, W. H. (1972) *Aust. J. Biol. Sci.* **25**, 949–961
- McKenzie, H. A., Ralston, G. B. & Shaw, D. C. (1972) *Biochemistry* **11**, 4539–4547
- Munk, P. & Cox, D. J. (1972) *Biochemistry* **11**, 687–697
- Perlmann, G. E. & Longworth, L. G. (1948) *J. Am. Chem. Soc.* **70**, 2719–2724
- Roark, D. E. & Yphantis, D. A. (1969) *Ann. N.Y. Acad. Sci.* **164**, 245–278
- Steiner, R. F. (1952) *Arch. Biochem. Biophys.* **39**, 333–354
- Tanford, C., Bunville, L. G. & Nozaki, Y. (1959) *J. Am. Chem. Soc.* **81**, 4032–4036
- Teller, D. C. (1973a) *Methods Enzymol.* **27**, 372–373
- Teller, D. C. (1973b) *Methods Enzymol.* **27**, 392
- Teller, D. C. (1973c) *Methods Enzymol.* **27**, 392–397
- Teller, D. C., Horbett, T. A., Richards, E. G. & Schachman, H. K. (1969) *Ann. N.Y. Acad. Sci.* **164**, 66–101
- Williams, J. W., van Holde, K. E., Baldwin, R. L. & Fujita, H. (1958) *Chem. Rev.* **58**, 715–806
- Yphantis, D. A. (1964) *Biochemistry* **3**, 297–317
- Zimmerman, J. K., Barlow, G. H. & Klotz, I. M. (1970) *Arch. Biochem. Biophys.* **138**, 101–109