Thermochimica Acta, 159 (1990) 305–325
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

BIOLOGICAL THERMODYNAMIC DATA FOR THE CALIBRATION OF DIFFERENTIAL SCANNING CALORIMETERS: HEAT CAPACITY DATA ON THE UNFOLDING TRANSITION OF β-LACTOGLOBULIN IN SOLUTION

FREDERICK P. SCHWARZ

Center for Advanced Research in Biotechnology, National Institute of Standards and Technology, Gaithersburg, MD 20899 (U.S.A.)

(Received 14 August 1989)

ABSTRACT

Differential scanning calorimetry (DSC) measurements of the unfolding transition of β-lactoglobulin in HCl–glycine-buffered solutions have been performed over a temperature range from 353.0 K at pH 2.3 to 362.2 K at pH 3.5. Three values for the van't Hoff transition enthalpies were calculated as follows: (1) from the fit of a two-state transition model to the heat capacity measurements (ΔH_{vf}); (2) from the van't Hoff plot of $\ln(1/K)$ $1/T$ where K is the transition equilibrium constant; and (3) from the equation $\Delta H_{ve} = 4.00RT_m^2C_p^{max}/A_p$ where $R = 8.31451$ J mol^{-1} K^{-1}, T_m is the transition temperature, C_p^{max} is the peak maximum, and A_p is the peak area. The best linear fit of the van't Hoff enthalpies to the transition temperatures, T_m, was obtained with ΔH_{vf} and is ΔH_{vf} (kJ mol^{-1}) $= (438.6 \pm 2.8) + (1.8 \pm 1.0)(T_m - 358.0)$. Calorimetric transition enthalpies were determined from the transition peak area using an extrapolated sigmoidal baseline and using an extrapolated straight baseline. The best linear dependence of the calorimetric enthalpy on T_m was obtained with the sigmoidal baseline (ΔH_s) and is ΔH_s(kJ mol^{-1}) $= (427.1 \pm 4.2) + (0.5 \pm 1.5)(T_m - 358.0)$. Linear least-squares fits of ΔH_{vf} and ΔH_s to T_m were independent of the DSC scan rate, the source of β-lactoglobulin, the buffer concentration from 0.1 to 0.2 M, and the concentration of the protein from 0.03 to 0.45 mM. The transition temperature exhibits a linear dependence on pH and a slight dependence on concentration. Cooperativity of the transition is 0.974 ± 0.007 while the average heat capacity change of the solution accompanying the transition is 13.5 ± 7.8 kJ mol^{-1} K^{-1} over this temperature range.

INTRODUCTION

Heat capacity data on the unfolding transition of ribonuclease a [1] and lysozyme [2] in HCl–glycine-buffered solutions have been reported for use in the calibration and testing of differential scanning calorimeters (DSCs) specifically designed for microcalorimetry measurements on 1 ml samples of aqueous solutions. Transition enthalpies, temperatures, cooperativities, and solution heat capacity changes in HCl–glycine-buffered solutions were reported over a transition temperature range from 312 K (pH 2) to 335 K (pH

4) for ribonuclease a, and from 326 K (pH 2.3) to 349 K (pH 3.9) for lysozyme. To extend the range of heat capacity data to near the usual maximum operating temperature of most DSCs (373 K), the unfolding transition of the more thermally stable protein, bovine β-lactoglobulin, was similarly investigated.

In the ribonuclease a and lysozyme investigations, two commonly employed methods were used to determine the van't Hoff enthalpies. Van't Hoff enthalpies were determined from a fit of a two-state transition model to the data [1] and from a van't Hoff plot of ln K vs. $1/T$, where K is the transition equilibrium constant and T is the temperature. For the lysozyme transitions, van't Hoff enthalpies were also calculated from the ratio of the maximum transition peak height, C_p^{max}, to the peak area, A_p, through the relationship

$$\Delta H_{ve} = 4.00 R T_m^2 C_p^{max} / A_p \quad (1)$$

where $R = 8.31451$ J mol^{-1} K^{-1} and T_m is the transition temperature. Calorimetric enthalpies were determined from the area under the transition profile with an extrapolated sigmoidal baseline and a straight baseline which is sometimes used to calculate the calorimetric enthalpy. All the transition enthalpies exhibited a linear dependence on temperature while the transition temperatures exhibited a linear dependence on pH and a slight linear dependence on concentration [1,2]. The cooperativities of the ribonuclease a transitions were slightly greater than unity, e.g. 1.057 ± 0.014 at 333.2 K, and exhibited a slight linear dependence on temperature [1], while the cooperativities of the lysozyme transitions were 1.007 ± 0.008 at 333.2 K and exhibited a smaller dependence on temperature [2]. The results were independent of the source of the proteins, the DSC scan rate, and the buffer concentration from 0.1 to 0.2 M [1,2].

Bovine β-lactoglobulin is the major whey protein in milk [3] and occurs in two different forms, β-lactoglobulin A and β-lactoglobulin B. β-lactoglobulin A, which has two more carboxyl groups than β-lactoglobulin B per molecule of molecular mass 36 kDa, aggregates and exhibits an isoelectric point different from that of β-lactoglobulin B which does not aggregate [4]. Commercial β-lactoglobulin consists of a mixture of these two forms in varying proportions. The thermal unfolding transition of β-lactoglobulin in aqueous solutions has been studied by UV spectroscopy from pH 1 to 7.5 [5] and by differential scanning calorimetry at pH 2.9 [6], 3.0–10.0 [7], and 6.5 [3]. The UV measurements were performed on non-buffered aqueous solutions of the protein adjusted to the appropriate pH by the addition of acid and exhibited a decrease in the denaturation temperature with increase in pH from 356.5 K at pH 1.5 to 353.3 K at pH 3.0. The DSC results, however, exhibited a transition temperature of 358 K at pH 3.0 in HCl–water solutions [7] and a transition temperature of 363 K at pH 2.9 in HCl–glycine-buffered solutions [6]. In this study, the transition properties of

β-lactoglobulin from four different sources were investigated as a function of concentration, pH, DSC scan rate, and buffer concentration. The transition properties were the transition temperature, enthalpies, cooperativities, and heat capacity changes of the solution accompanying the transition. The results were compared with the results from the unfolding transitions of pure β-lactoglobulin A and β-lactoglobulin B in solution. The pH range was limited to values below 3.5 to avoid aggregation of the protein which occurs over the pH range 3.5–5.2 [8]. Calorimetric and van't Hoff enthalpies similar to those calculated for the unfolding transition of lysozyme were determined and compared to ascertain the suitability of the transition enthalpies of β-lactoglobulin in solution for the calibration of DSCs in the temperature range from 353 to 362 K. These heat capacity data along with those of ribonuclease a and lysozyme would thus provide a complete set of enthalpies of well-characterized unfolding transitions for the calibration of DSCs from 312 to 362 K.

EXPERIMENTAL

Materials

Samples of bovine β-lactoglobulin were obtained from four different commercial sources and contained different amounts of the A and B forms. Samples of the pure A form and the pure B form were also obtained from one of the commercial sources. The hydrochloric acid, phosphoric acid, acetic acid, glycine, sodium acetate, sodium phosphate, and sodium chloride were reagent quality.

Preparation and analysis of solutions

Solutions at a concentration of approximately 0.5 mM were prepared by dissolving 0.2 g of β-lactoglobulin in 10 ml of 0.2 M HCl–glycine buffer solution at pH 3.0. The glycine buffer was prepared by dissolving 0.2 mol of glycine in 1 l of distilled water and adding concentrated HCl dropwise with stirring until the solution reached the desired pH as monitored with an Orion 811 * pH meter operated with a Corning EX-L glass electrode. The solutions were dialyzed using a membrane with a molecular weight cut-off of

* Certain commercial equipment, instruments and materials are identified in this paper in order to specify the experimental procedure as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the material, instrument, or equipment identified is necessarily the best available for the purpose.

3500 against a volume of about 400 ml of the buffer solution which was replaced twice during the course of the dialysis. The solutions were stored at room temperature in the dark for up to one week. Prior to the DSC measurement, a 2 ml aliquot of solution was prepared at a specific concentration and pH by diluting the dialyzed solution with buffer solution at an appropriate pH. A portion of this solution was set aside for analysis by UV spectroscopy. The spectroscopic analysis consisted of diluting the sample to a concentration of approximately 10 μM with 0.1 M sodium acetate buffer at pH 4.0 and measuring the optical density at 280 nm with a Perkin–Elmer Lambda 4B spectrophotometer. An extinction coefficient of 3.46×10^4 l mol^{-1} cm^{-1} was calculated from an absorptivity of 0.96 l g^{-1} cm^{-1} at 278 nm in 0.1 M sodium acetate buffer at pH 5.3 [8] and a molar mass of 36 kDa for β-lactoglobulin [4] (the absorptivity at 278 nm was found to be the same at pH 4.0). The optical densities were measured at pH 4 to minimize any possibility of aggregation of the more concentrated samples. The density of the 0.1 M sodium-acetate-buffered solutions was determined by weighing known volumes of the solutions and was 1.005 ± 0.001 g ml^{-1}, while that of the 0.2 M HCl–glycine-buffered solutions was 0.994 ± 0.002 g ml^{-1} at room temperature.

To ascertain the purity of the β-lactoglobulin, 1 mass% 0.05 M sodium-phosphate-buffered solutions at pH 5.8 were prepared from each of the four commercial sources and analyzed using a Waters 650E HPLC. 50 μl samples of the solutions were analyzed by passage, at a flow rate of 0.1 ml min^{-1}, through a Waters 300 SW gel filtration column (15 cm × 3.9 mm) connected to a Waters 484 UV absorbance detector operated at 280 nm. The resolution of the column was such that a minimum molecular weight difference of 5 kDa could be resolved. The samples were also analyzed by passage through a Waters DEAE-5PW anion exchange column substituted for the 300 SW column to determine the amount of β-lactoglobulin A and B in each sample. This procedure was similar to a procedure for chromatographic separation of β-lactoglobulins A and B developed by Piez et al. [4]. For elution through the column, a linear gradient was established by replacing the 0.05 M sodium phosphate buffer elutant (100% at zero time) with a solution of 0.08 M sodium chloride–0.05 M sodium phosphate buffer (100% at time 30 min) over the first 30 min at a flow rate of 1 ml min^{-1}. The elution of the A component was completed by flowing the sodium chloride–sodium phosphate solution through the column for an additional 30 min. The sample size was 0.3 ml and the column was 7 cm × 8 mm. The A and B component peaks of the resulting chromatograms were identified by comparison with chromatograms of solutions of the pure A and B components. Known concentrations of samples of the pure A and B solutions and the peak areas of their chromatograms were used to determine the sensitivity factors (area/mass) of A and B. These factors were then used to determine the mass ratio of the A to B components in each of the commercial sources.

DSC measurements

DSC measurements were performed with a Hart 7707 differential heat conduction scanning microcalorimeter as described previously [1,2]. During one scan, three sample cells containing 0.501 g of solution were scanned against a reference cell containing an equal mass of the buffer solution. The temperature and power calibration of all three of the DSC cells were performed in the same manner, as described in detail previously [1]. The DSC was normally operated at a scan rate of 20 K h^{-1} from 303.2 to 378.2 K. An excess power vs. temperature scan for the β-lactoglobulin transition was obtained by subtracting the power input of a thermal scan of buffer vs. buffer from the power input of a scan of the solution vs. buffer. All the excess-power thermal scans were corrected for the thermal lag of the DSC by the Tian equation and converted to excess heat capacity vs. temperature scans by dividing by the scan rate as described previously [1].

To determine the transitional baseline, temperature, van't Hoff transition enthalpy, and the change in the excess heat capacity of the baseline, the excess heat capacity vs. temperature profiles were fitted to the two-state transitional model described by Schwarz and Kirchhoff [1]. During the fitting procedure, the number of moles of protein was fixed at a value determined by the spectroscopic analysis. The sigmoidal transitional baseline was detected from extrapolations of the pre- and post-transitional baselines to the midpoint of the transition and the fractional area (α) under the transition profile at a given temperature [1]. The temperature of the transition, T_m, was the temperature at $\alpha = 0.5$. The change in the excess heat capacity of the baselines, ΔC_p, was recorded as the difference between the pre- and post-transtional baselines at T_m.

RESULTS AND DISCUSSION

Purity analysis

The chromatograms from the HPLC gel filtration column of the solutions exhibited only one peak at a mass of 35 ± 5 kDa, in agreement with the mass of 35 kDa for β-lactoglobulin. Under the operating conditions of the column, the minimum resolution was 5 kDa. The elutions were continued until two column volumes of the solution were eluted.

Retention times of the A and B β-lactoglobulins eluted from the DEAE anion exchange columns under the gradient conditions were, respectively, 54.3 min and 33.5 min. By comparing their sensitivity factors at 54.3 and 33.5 min, the ratio of component A to B in commercial source c was 1, in source d was 1.3, in source e was 1.1, and in source f was 1.5. In the chromatograms for sources c and f, an additional smaller peak with an area

less than 5% of the B component peak area was observed at a retention time of 39.2 min. Assuming that this peak has the same sensitivity factor as the B component peak, c and f were at least 95%, by mass, pure A and B. Similarly the anion exchange column results show that the samples from sources d and e were close to a 100% pure mixture of the A and B components.

DSC measurements

Typical thermal scans of β-lactoglobulin (0.3 mM) in 0.2 M HCl–glycine-buffered solutions are shown in Fig. 1 along with the computer-simulated excess heat capacity curve from the two-state model. The unfolding transition consists of a single symmetrical peak followed by an increase in the transitional baseline. An increase in the pH of the solution from 2.6 to 3.5 produces an increase in the transition temperature and very little change in the area under the transition profile. Both the pre-transitional and post-transitional baselines increase linearly with temperature, which has been observed for the unfolding of globular proteins such as ribonuclease a and lysozyme [1,2,9]. Extrapolation of the baselines to the transition temperature shows a positive increase from the pre-transitional to the post-transitional baseline, indicating an increase in the heat capacity of the solution, ΔC_p, upon denaturation which is also observed for the globular proteins [9].

A repeated thermal scan of a sample exhibited similar transition profiles with a decrease in the transition enthalpy. The extent of the decrease

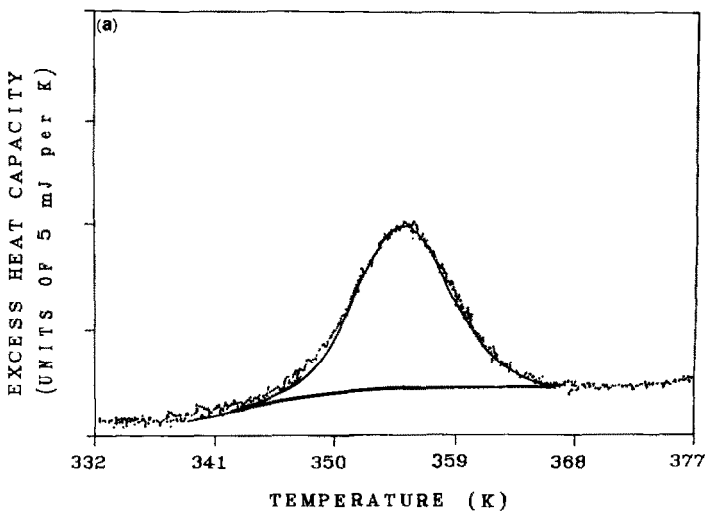

Fig. 1(a). DSC thermal scan number 22 in Table 1 (in 0.2 M HCl–glycine buffer at pH 2.6) at a scan rate of 20 K h^{-1}: ———, two-state model curve; ·····, experimental data. The sigmoidal baseline was generated by the two-state model.

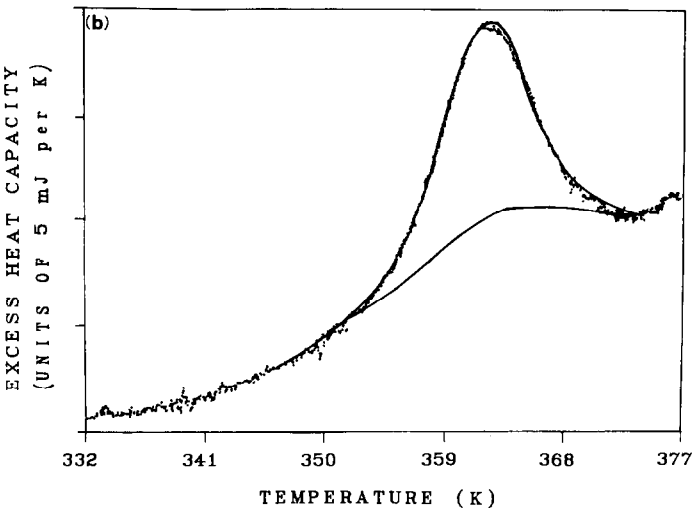

Fig. 1(b). DSC thermal scan number 67 in Table 1 (in 0.2 M HCl–glycine buffer at pH 3.3) at a scan rate of 20 K h^{-1}: ———, two-state model curve; ·····, experimental data. The sigmoidal baseline was generated by the two-state model.

depends on the pH of the solution, the temperature range, and rate of the thermal scan. A repeated scan of a transition occurring at 359 K which was initially scanned at 60 K h^{-1} from 333 to 369 K, resulted in a 30% decrease in the area under the transition profile. However, a repeated scan of a transition occurring at 352 K which was initially scanned at a slower rate of 20 K h^{-1} from 333 to 364 K, resulted in an 80% decrease in the area. Apparently there is thermal degradation of the β-lactoglobulin at high temperatures similar to that observed for ribonuclease a [1] and lysozyme [2] in solution. Zale and Klibanov [10] showed that denatured ribonuclease a undergoes irreversible hydrolysis of the peptide bonds at the aspartic acid residues and deamination of the asparagine and/or glutamine residues at 363 K and pH 4. It appears that thermal degradation is responsible for the lack of total reversibility of the transition. The dependence of the degradation on scan rate shows that the degradation proceeds at a slower rate than that of the unfolding transition, which will be shown below to be independent of scan rate. Only the results for the first thermal scan are reported for the samples studied.

Thermodynamic information on the unfolding transition of β-lactoglobulin in 0.1–0.2 M HCl–glycine buffer determined from DSC scans of 85 samples is summarized in Table 1. The β-lactoglobulin was from four different commercial sources designated by the letters c, d, e, and f. The leters a and b designate the A and B components of the protein which were obtained from source d. Samples were scanned at 20 and 60 K h^{-1} in three cells (1,2, and 3) which have different calibration factors. The samples scanned at 60 K h^{-1} are noted with an asterisk in Table 1. Most of the

TABLE 1

Thermodynamic properties of the β-lactoglobulin transition in HCl–glycine-buffered solution

Scan no.	Sample ID [a]	Conc. (10^{-4} M)	pH	ΔC_p (kJ mol^{-1} K^{-1})	C_p^{max} (kJ mol^{-1} K^{-1})	T_m (K)	ΔH_{vf} [b] (kJ mol^{-1})	ΔH_{ve} (kJ mol^{-1})	ΔH_{vs} (kJ mol^{-1})	ΔH_s (kJ mol^{-1})	ΔH_l (kJ mol^{-1})	$\Delta H_s / \Delta H_{vf}$
1	b2	2.67	2.27	22.5	55.9	352.9	483	481	491	481	561	1.00
2	d3	3.15	2.3	10.8	52.8	353.8	474	454	455	484	414	1.02
3	d1	3.15	2.3	2.29	45.2	354.5	435	462	457	409	601	0.94
4	e2	3.25	2.4	4.17	47.6	354.4	431	520	495	382	265	0.89
5	c3	3.17	2.42	11.06	45.3	354.0	438	442	447	426	471	0.97
6	d2	3.23	2.43	6.04	48.0	354.4	448	505	504	397	394	0.89
7	d3	3.23	2.43	0.53	49.1	354.9	472	454	476	453	407	0.96
8	e3 *	3.45	2.5	1.03	45.3	356.9	445	414	414	464	379	1.04
9	e1 *	3.45	2.5	3.07	42.0	357.2	429	409	412	436	501	1.02
10	c3 *	3.98	2.57	23.1	48.3	356.0	445	476	480	427	479	0.96
11	c1	2.00	2.58	7.33	50.1	355.9	461	452	443	466	621	0.96
12	c2	2.00	2.58	1.42	46.5	356.1	428	486	446	404	832	1.01
13	d1 *	4.14	2.58	18.6	42.8	356.8	427	429	442	422	737	0.99
14	c2	0.97	2.58	18.8	50.0	355.0	463	406	415	516	500	1.11
15	e1	0.69	2.58	4.87	57.1	354.6	447	472	461	423	255	0.95
16	e1	1.60	2.58	5.55	44.1	355.0	442	420	435	440	247	1.00
17	d1 *	4.14	2.59	8.46	43.9	356.8	434	429	436	433	617	1.00
18	d2 *	4.14	2.59	4.56	41.5	357.0	412	441	442	399	541	0.97
19	d3	1.78	2.59	9.12	52.2	355.9	460	438	419	502	532	1.09
20	d2	1.78	2.59	0	52.2	355.4	465	472	447	464	437	1.00
21	d1	0.95	2.59	24.4	44.6	355.1	411	419	404	446	710	1.09
22	e1	3.37	2.59	1.53	47.8	354.9	446	432	417	464	483	1.04
23	e3	3.37	2.59	0	45.6	355.2	436	464	452	412	385	0.94
24	e1	3.45	2.6	6.71	40.6	355.2	395	472	461	361	644	0.91
25	e2	3.45	2.6	15.3	39.2	354.5	400	445	466	368	507	0.92
26	e3	3.45	2.6	4.21	43.7	355.5	440	437	455	420	429	0.95
27	f1	1.69	2.6	17.5	38.7	355.1	384	446	421	364	490	0.95
28	f2	1.69	2.6	21.2	51.8	354.3	415	446	438	400	216	0.96
29	c1	2.83	2.6	27.1	48.6	354.5	434	469	458	433	783	1.00

30	d3	2.81	2.6	20.4	51.3	354.8	441	510	530	385	439	0.87
31	d1	2.81	2.6	17.8	51.9	355.5	461	487	473	448	570	0.97
32	c2	3.35	2.69	11.6	44.4	355.9	430	467	471	401	506	0.93
33	c3	3.35	2.69	28.2	42.2	355.0	401	468	471	378	563	0.94
34	a2	3.05	2.8	15.1	40.4	356.4	423	418	441	408	331	0.96
35	c2	3.52	2.8	15.8	45.8	356.5	436	453	444	428	448	0.98
36	c3	3.52	2.8	6.1	40.6	357.7	409	468	453	369	730	0.90
37	a1	3.05	2.83	23.3	38.8	356.6	394	412	411	398	534	1.01
38	c1	2.42	2.83	11.5	44.2	356.7	431	430	435	434	639	1.01
39	c1/2	3.11	2.92	20.6	42.6	356.5	439	407	439	442	617	1.01
40	c3/2	3.11	2.92	17.2	47.5	356.5	443	451	447	445	615	1.01
41	e2	3.84	3.00	21.5	40.1	356.9	405	332	397	512	918	1.26
42	e1	1.58	3.02	22.3	35.1	357.4	407	383	447	390	323	0.96
43	e2	1.58	3.02	21.8	45.0	357.2	455	441	465	433	184	0.95
44	e1/2	3.62	3.10	7.34	40.1	358.6	404	448	442	383	590	0.95
45	e3/2	3.62	3.10	23.8	41.4	357.6	401	477	470	369	485	0.92
46	b3	4.46	3.19	5.8	44.4	359.4	444	462	473	412	262	0.93
47	b1	4.46	3.19	10.9	38.8	359.1	398	458	456	364	445	0.91
48	f1	3.19	3.19	18.4	38.9	358.9	400	429	425	389	561	0.97
49	f2	3.19	3.19	8.71	36.0	359.6	423	376	427	412	399	0.97
50	d2	3.07	3.24	12.7	54.3	360.2	478	488	472	480	652	1.00
51	a1	3.05	3.26	16.4	38.8	359.7	415	387	400	431	614	1.04
52	c1 *	3.80	3.28	21.5	49.8	362.0	455	532	531	408	568	0.90
53	f1	1.78	3.28	7.15	40.0	361.5	412	484	476	359	340	0.87
54	f3	1.78	3.28	18.2	44.9	360.4	428	436	410	444	420	1.04
55	c3 *	3.80	3.28	27.1	53.1	361.6	471	511	514	452	552	0.96
56	d1 *	1.35	3.28	3.52	45.3	362.7	453	442	447	449	568	0.99
57	d1	0.67	3.28	5.44	37.4	361.6	487	511	494	465	309	0.95
58	d3	0.67	3.28	34	55.6	360.1	475	505	515	412	435	0.87
59	e1	1.35	3.28	22.4	35.3	360.4	425	365	452	418	515	0.98
60	d3	1.35	3.28	3.16	49.1	361.5	460	468	449	456	596	0.99
61	c1	1.88	3.28	31.7	46.9	359.4	446	489	518	413	385	0.93
62	c2	1.88	3.28	14.7	52.3	360.4	489	496	523	456	396	0.93
63	c3	1.88	3.28	23.6	56.8	360.2	482	536	515	458	471	1.01
64	c3	0.95	3.28	24.1	55.4	360.6	468	511	467	469	725	1.00

TABLE 1 (continued)

Scan no.	Sample ID [a]	Conc. (10^{-4} M)	pH	ΔC_p (kJ mol^{-1} K^{-1})	C_p^{max} (kJ mol^{-1} K^{-1})	T_m (K)	ΔH_{vf} [b] (kJ mol^{-1})	ΔH_{ve} (kJ mol^{-1})	ΔH_{vs} (kJ mol^{-1})	ΔH_s (kJ mol^{-1})	ΔH_l (kJ mol^{-1})	$\Delta H_s / \Delta H_{vf}$
65	f3	0.89	3.28	2.07	48.0	361.2	469	478	483	436	391	0.93
66	c1	3.13	3.29	4.13	45.6	360.8	440	477	465	414	714	0.94
67	c2	3.13	3.3	19.3	46.0	360.0	444	463	468	428	684	0.96
68	d3 *	1.35	3.3	3.94	45.0	363.1	446	472	481	418	506	0.94
69	c3	0.33	3.3	41.6	63.3	360.3	447	538	447	508	621	1.14
70	c1	0.33	3.31	25.1	56.8	360.1	418	473	395	518	1201	1.24
71	e2	3.84	3.31	5.64	41.2	360.7	413	483	469	369	613	0.89
72	e3	3.84	3.4	3.14	44.9	361.0	432	474	464	411	504	0.95
73	c1	3.31	3.49	1.29	49.0	362.3	460	484	474	442	677	0.96
74	c2	3.31	3.49	11.9	51.6	361.9	473	491	486	457	605	0.97
75	c3	3.31	3.49	13.2	51.3	361.9	465	499	486	448	623	0.96
76	e1	3.66	3.5	1.2	43.0	361.7	431	476	486	392	632	0.91
77	e2	3.66	3.5	6.53	44.1	361.3	435	465	461	412	638	0.95
78	f1	3.96	3.52	5.37	38.6	362.4	405	425	417	396	520	0.98
79	d1	3.07	3.52	12.8	58.3	362.0	498	526	513	483	634	0.97
80	d3	3.07	3.52	12.7	53.5	361.9	503	498	535	468	576	0.92
81	e1/2	3.68	3.53	8.22	42.3	360.9	426	449	460	408	629	0.96
82	c2/2	3.52	3.53	8.05	45.2	361.7	444	461	466	427	663	0.94
83	e3/2	3.68	3.53	0	38.2	361.4	418	471	472	352	559	0.84
84	b2	4.10	3	7	47.3	358.7	446	454	448	445	452	1.00
85	a1	2.83	3.56	12.3	39.7	361.6	408	417	403	414	592	1.01

[a] The sample is described by a letter a or b (for component A and B) or c–f for the source of β-lactogloublin, by a number which designates which of the three DSC sample cells were used for the measurements, by /2 if the buffer was 0.1 M instead of the usual 0.2 M, and by an asterisk if the sample was scanned at 60 K h^{-1} instead of the normal 20 K h^{-1}. The sample size was 0.501 g of solution and the sample was scanned from 313.2 to 388.2 K.

[b] The transition enthalpies ΔH_{vf}, ΔH_{ve}, ΔH_{vs}, ΔH_s, and ΔH_l were determined as follows: from the fit of a two-state transition model to the heat capacity measurements (ΔH_{vf}); from the van't Hoff plot of $\ln(1/K)$ vs. $1/T$ where K is the transition equilibrium constant (ΔH_{vs}); from the equation $\Delta H_{ve} = 4.00\, RT_m^2 C_p^{max}/A_p$ where $R = 8.31451$ J mol^{-1} K^{-1}, T_m is the transition temperature, C_p^{max} is the peak maximum, and A_p is the peak area; from the area of the peak with the sigmoidal baseline (ΔH_s); and from the area of the peak with a straight baseline (ΔH_l).

solutions were 0.2 M HCl–glycine and those solutions at 0.1 M HCl–glycine buffer concentrations are designated as /2. The concentrations were determined from spectroscopic analysis of the solutions and used to determine van't Hoff transition enthalpies from the fit (ΔH_{vf}) and the calorimetric transition enthalpies using the sigmoidal baseline (ΔH_s) and a linear baseline (ΔH_l). The van't Hoff enthalpy from the fit is corrected for temperature variation over the temperature range of the transition peak [1]. A value for the van't Hoff enthalpy at the transition temperature, ΔH_{ve}, was calculated from eqn. (1) with A_p equal to the area under the peak with the sigmoidal baseline. A van't Hoff enthalpy without the temperature correction, ΔH_{vs}, was calculated from a traditional van't Hoff plot described briefly as follows. The fraction of protein in the denatured state, $\theta(T)$, was taken as the ratio of the fractional area of the transition curve up to a temperature T over the total area of the curve above the sigmoidal baseline. The equilibrium constant is then $K(T) = \theta(T)/(1 - \theta(T))$ and ΔH_{vs} is obtained from the slope of ln K vs. $1/T$

$$\mathrm{d} \ln K/\mathrm{d}T^{-1} = -\Delta H_{vs}/R \tag{2}$$

The cooperativity of the transition, η, was taken as the ratio of ΔH_s to the temperature-corrected van't Hoff enthalpy determined from a fit of the two-state model to the data, ΔH_{vf}. All five transition enthalpies were analyzed as linear functions of the transition temperature and the transition temperature was analyzed as a linear function of the pH.

Transition temperature dependence on pH and concentration of β-lactoglobulin

A plot of the transition temperature as a function of pH is shown in Fig. 2 along with the linear least-squares fit of the data. Over the pH range 2.3–3.5 for the HCl–glycine buffer, the transition temperature increased with pH according to the following equation:

$$T_m = 337.5 \pm 0.8 + (6.95 \pm 0.25)\mathrm{pH} \tag{3}$$

with a standard deviation of 0.9 K. A transition temperature of 358.4 K is calculated at pH 3.0 from eqn. (4), in agreement with the transition temperature of 358 K reported from the DSC results of Paulsson et al. [7]. A similar increase in the transition temperature with pH has also been observed with lysozyme [2], ribonuclease a [1] and other globular proteins [9]. This increase in the stability of the protein is brought about by a decrease in its net positive charge as the solution becomes less acidic [11].

The change in proton binding between the final, unfolded state, n_f, and the initial, folded state, n_i, of β-lactoglobulin in solution can be determined from the slope of eqn. (3) because [12]

$$n_f - n_i = \left(\Delta \mathrm{H}_s/2.303RT_m^2\right)\, \partial(T_m)/\partial(\mathrm{pH}) \tag{4}$$

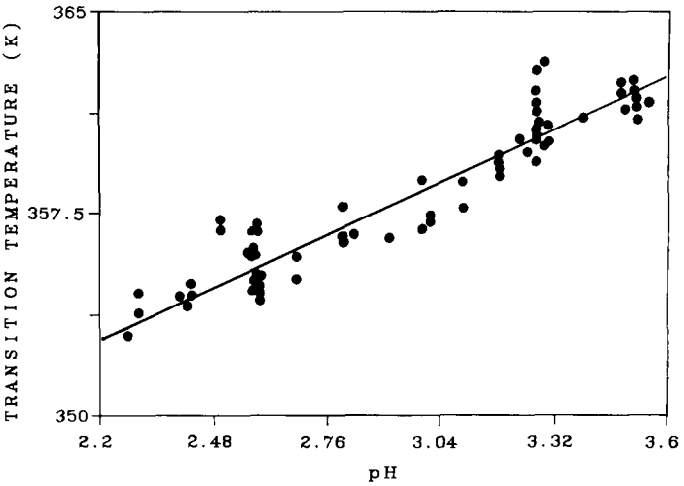

Fig. 2. Plot of the transition temperature vs. pH and the linear least-squares fit of T_m to pH, $T_m(K) = 337.5 \pm 0.8 + (6.95 \pm 0.25)pH$.

At the intermediate temperature of 358 K, $\Delta H_s = 427$ kJ mol^{-1}, yielding $n_f - n_i = 1.2$ and thus 1.2 protons are absorbed per molecule of β-lactoglobulin as it unfolds at this temperature. This is half the value of 2.2 protons per molecule of ribonuclease a [11] and 2.6 protons per molecule of lysozyme [2]. As the heat of ionization of glycine is about 2.51 kJ mol^{-1} [11], this would only contribute about 0.5% to the transition enthalpies which is less than the experimental error.

There is also a slight dependence of transition temperature on concentration of the enzyme as shown by the deviation of the points clustered at pH 2.6 in Fig. 2. At pH 2.6 the concentration of the samples ranged from 0.095 to 0.414 mM and there is an increase in transition temperature with concentration of $3.4 \pm 1.4 \times 10^3$ K mol^{-1} l. Over nearly the same concentration range at pH 3.28, the increase, if any, is within the error in the determination ($2.1 \pm 2.5 \times 10^3$ K mol^{-1} l. This is in contrast with the decrease in T_m with increase in enzyme concentration observed for ribonuclease a [1] and lysozyme [2]. The increase in the transition temperature with concentration may be interpreted as a slight aggregation of the protein in the native state at pH 2.6.

Dependence of transition enthalpy on temperature

The values of the enthalpies ΔH_{vf}, ΔH_{ve}, ΔH_{vs}, ΔH_s, and ΔH_l are given in Table 1 and are plotted in Figs. 3–7 as a linear function of the transition temperature, i.e.

$$\Delta H_i = \Delta H_{i,0} + \Delta C'_{p,i}(T_m - 358.0) \qquad (i = \text{vf, ve, vs, s, l}) \qquad (5)$$

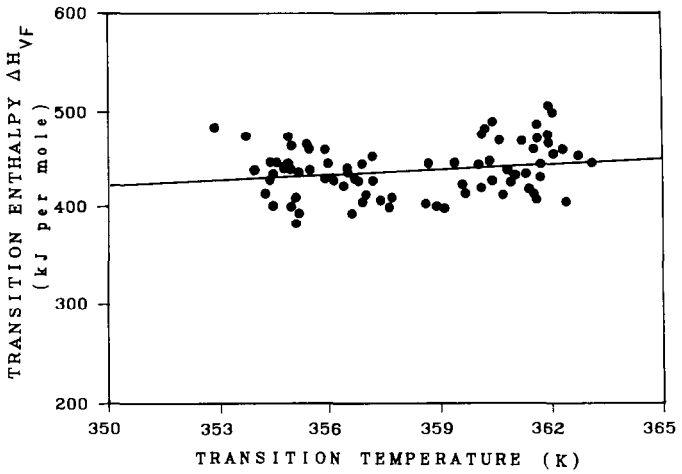

Fig. 3. Plot of ΔH_{vf} vs. the transition temperature and the linear least-squares fit of ΔH_{vf} to T_m, ΔH_{vf} (kJ mol^{-1}) = $438.6 \pm 2.8 + (1.8 \pm 1.0)(T_m - 358.0)$.

The enthalpies were fitted by the method of least squares with a reference temperature of 358.0 K rather than the conventional 298.2 K, since it falls at the midpoint of the temperature range of measurements. The results of these fits to all the data are presented in Table 2 and show a small linear dependence on temperature ($d(\Delta H_{vf})/dT_m = 1.8 \pm 1.0$ kJ K^{-1} mol^{-1}) as compared with the results of ribonuclease a ($d(\Delta H_v/dT_m = 3.6 \pm 1.9$ kJ K^{-1} mol^{-1} [1]) and lysozyme ($d(\Delta H_{vf})/dT_m = 5.81 \pm 0.24$ kJ K^{-1} mol^{-1} [2]). The van't Hoff enthalpies ΔH_{vf}, ΔH_{ve}, and ΔH_{vs} provide better linear fits of the enthalpy to the temperature, with standard deviations from 26.0 to

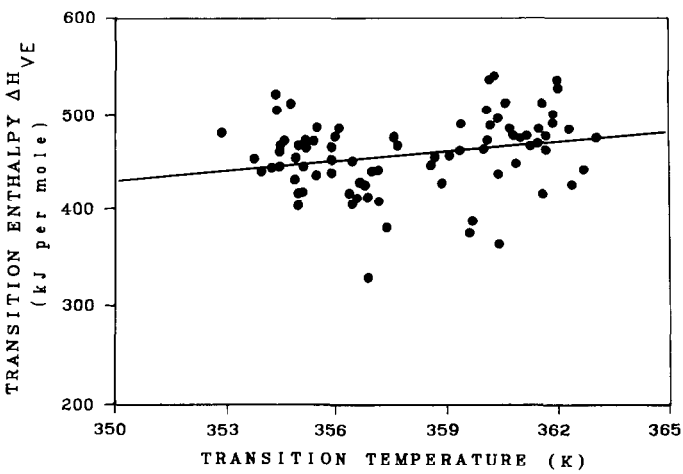

Fig. 4. Plot of ΔH_{ve} vs. the transition temperature and the linear least-squares fit of ΔH_{ve} to T_m, ΔH_{ve}(kJ mol^{-1}) = $458.3 \pm 4.1 + (3.4 \pm 1.4)(T_m - 358.0)$.

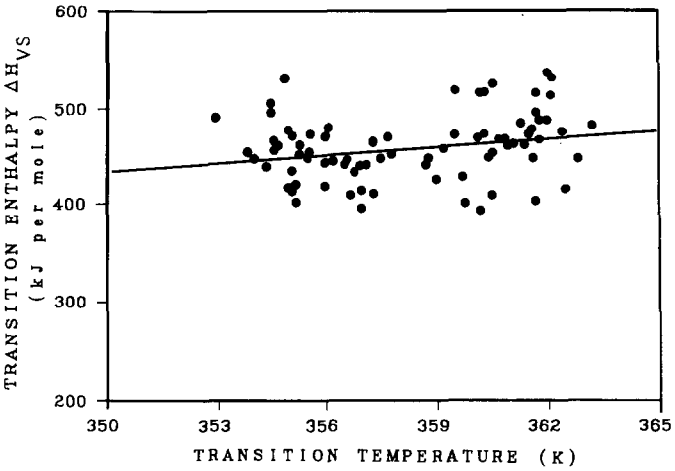

Fig. 5. Plot of ΔH_{vs} vs. the transition temperature and the linear least-squares fit of ΔH_{vs} to T_m, $\Delta H_{vs}(\text{kJ mol}^{-1}) = 458.0 \pm 3.5 + (2.9 \pm 1.2)(T_m - 358.0)$.

37.7 kJ mol^{-1}, than the calorimetric enthalpies, ΔH_s and ΔH_l, with standard deviations of 38.6 and 144 kJ mol^{-1}, respectively. In comparing the linear dependences of the van't Hoff enthalpies on temperature, the best fit is obtained with the temperature-corrected van't Hoff enthalpy from the fit of the two-state transition model to the data, ΔH_{vf}. In addition, the intercept ΔH_0 for $\Delta H_{vf} = \Delta H_0 + \Delta C_p'(T_m - 358.0))$ is 2.7% greater or within $3 \times \sigma(\Delta H_0)$ of ΔH_0 for $\Delta H_s = \Delta H_0 + \Delta C_p'(T_m - 358.0))$ while the $\Delta C_p'$ values are the same, i.e. the linear dependence of ΔH_{vf} on T_m is within three standard deviations of the linear dependence of ΔH_s on T_m.

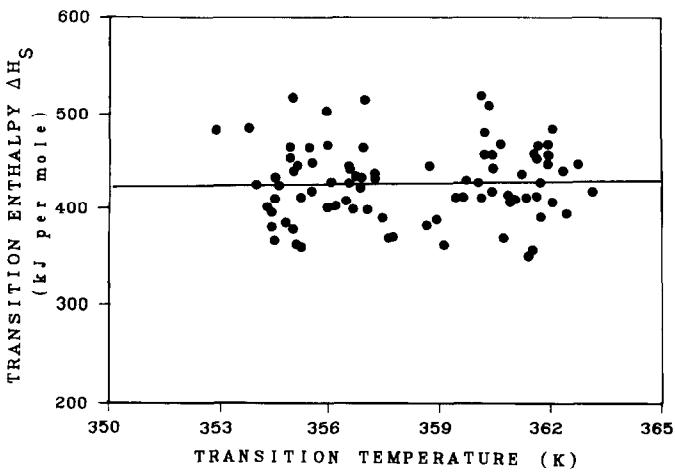

Fig. 6. Plot of ΔH_s vs. the transition temperature and the linear least-squares fit of ΔH_s to T_m, $\Delta H_s(\text{kJ mol}^{-1}) = 427.1 \pm 4.2 + (0.5 \pm 1.5)(T_m - 358.0)$.

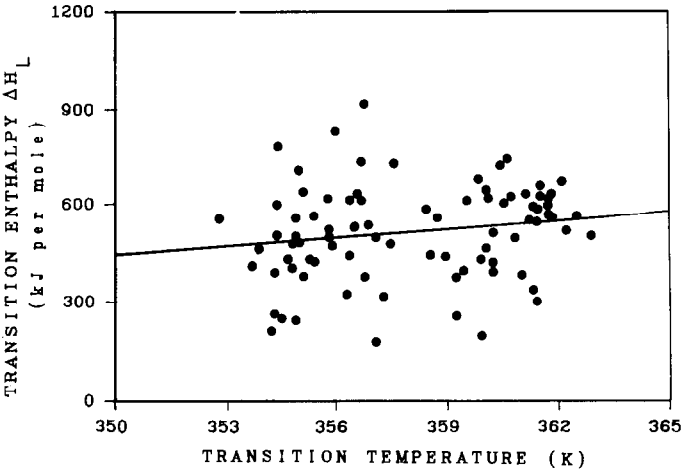

Fig. 7. Plot of ΔH_l vs. the transition temperature and the linear least-squares fit of ΔH_l to T_m, $\Delta H_l(\text{kJ mol}^{-1}) = 518 \pm 16 + (8.9 \pm 5.5)(T_m - 358.0)$.

The linear dependence of ΔH_{ve} on T_m is well within a standard deviation of the linear dependence of ΔH_{vs} on T_m, as expected, since ΔH_{ve} from eqn. (2) is the value of $R\ \mathrm{d}(\ln\{K_e\})/\mathrm{d}T^{-1}$ from eqn. (3) at the midpoint of the transition. In Table 2, the intercepts of the fits of ΔH_{ve} and ΔH_{vs} to T_m are 4% or five standard deviations higher than the intercept of the fit of ΔH_{vf} to T_m, which could be accounted for by including the UV spectroscopic analysis results in the calculation of ΔH_{vf}. The calculation of ΔH_{ve} and

TABLE 2

Transition enthalpies of β-lactoglobulin in 0.1–0.2 M HCl–glycine-buffered solutions as a function of transition temperature

Transition enthalpy [a] (kJ mol^{-1})	Result of linear fit to T_m $\Delta H = \Delta H_0 + \Delta C_p'(T_m - 358.0)$		
	ΔH_0 (kJ mol^{-1})	$\Delta C_p'$ (kJ K^{-1} mol^{-1})	σ (kJ mol^{-1})
ΔH_{vf}	438.6 ± 2.8	1.8 ± 1.0	26.0
ΔH_{ve}	458.3 ± 4.1	3.4 ± 1.4	37.7
ΔH_{vs}	458.0 ± 3.5	2.9 ± 1.2	31.9
ΔH_s	427.1 ± 4.2	0.5 ± 1.5	38.6
ΔH_l	518 ± 16	8.9 ± 5.5	144

[a] The transition enthalpies ΔH_{vf}, ΔH_{ve}, ΔH_{vs}, ΔH_s, and ΔH_l were determined as follows: from the fit of a two-state transition model to the heat capacity measurements (ΔH_{vf}); from the van't Hoff plot of $\ln(1/K)$ vs. $1/T$ where K is the transition equilibrium constant (ΔH_{vs}); from the equation $\Delta H_{ve} = 4.00 R T_m^2 C_p^{max}/A_p$ where $R = 8.31451$ J mol^{-1} K^{-1}, T_m is the transition temperature, C_p^{max} is the peak maximum, and A_p is the peak area; from the area of the peak with the sigmoidal baseline (H_s); and from the area of the peak with a straight baseline (ΔH_l).

ΔH_{vs} is based solely on the properties of the transition peak while the calculation of ΔH_{vf} is also based on the number of moles of protein in the sample which was determined by spectroscopic measurements. If, for example, the extinction coefficients were too low by 4%, then the amounts of protein in the cell would be calculated as 4% higher than they were and accordingly, the values of ΔH_{vf} and ΔH_s would be about 4% lower than their true values. However, there is no evidence to suggest that the extinction coefficient for the UV absorption by β-lactoglobulin at 278 nm is too low by 4%. Alternatively, neglecting a temperature variation in the determination of ΔH_{ve} and ΔH_{vs} could account for the 4% difference between these values and ΔH_{vf}. In eqn. (2), ΔH is assumed to be constant when, instead, it exhibits a temperature dependence as shown by the slopes, $\Delta C_p'$, of the fits of ΔH to T_m in Table 2. Not taking into account a temperature dependence could be responsible for the poorer fit of the ΔH_{ve} and ΔH_{vs} values to T_m. As with the lysozyme results, the best linear dependence on T_m is with the van't Hoff enthalpy, ΔH_{vf}, and for the calorimetric enthalpy, ΔH_s.

Paulsson et al. [7] reported a calorimetric enthalpy of 482 kJ mol^{-1} at 355 K which is in fair agreement with the ΔH_s value of 425 ± 6 kJ mol^{-1} obtained from eqn. (5). De Witt and Swinkels [3] reported a value for ΔH of 230 ± 15 kJ mol^{-1} at 343 K and pH 6.5 which was out of the pH range of the measurements reported here. This low value could result from aggregation of the β-lactoglobulin which is known to occur at this pH [8].

The heat capacity change of the solution

In Table 1, the change in the heat capacity of the solutions accompanying denaturation, ΔC_p, is the same within experimental error at all the transition temperatures. This was also the case for the denaturation of ribonuclease a [1] and lysozyme [2]. The average value of ΔC_p is 12.7 ± 9.2 kJ mol^{-1} K^{-1} which is within the range 4–7 kJ mol^{-1} K^{-1} obtained by Privalov and Khechinashvili [9] for globular proteins in HCl–glycine-buffered solutions.

Privalov and Khechinashvili [9] showed that, since $\Delta C_p = d(\Delta H)/dT$ for the globular proteins, the dependence of the transition enthalpy on temperature results from the difference in the heat capacities between the folded and unfolded states of the protein. All the $d\Delta H/dT_m$ ($\Delta C_p'$) values in Table 2, however, are close to zero, e.g. $d\Delta H_s/dT_m = 0.5 \pm 1.5$ kJ mol^{-1} K^{-1} and $d\Delta H_{vf}/dT_m = 1.8 \pm 1.0$ kJ mol^{-1} K^{-1}. The smaller $d\Delta H_{vf}/dT_m$ and $d\Delta H_s/dT_m$ values could be partly accounted for by the smaller temperature range of 11 K for the measurements as compared with a range of 23 K for the globular proteins [1,2,9]. Apparently the change in the heat capacity between the folded and unfolded states of β-lactoglobulin is not as evident in the transition enthalpy determinations as with the globular proteins.

Cooperativity of the transition

The two-state model is based on the assumption that the transition is fully cooperative, i.e. that the total protein unfolds as a single entity, and the observed calorimetric heat for the unfolding transition should be the same as the van't Hoff enthalpy. Actually Privalov and Khechinashvili [9] observed that the ratio of the calorimetric enthalpy to the van't Hoff enthalpy, the cooperativity, was 1.05 ± 0.03 for the globular proteins, indicating that the unfolding was not exactly two state but involved intermediate states in the denaturation. Since the ΔH_{vf} values have the best precision of the van't Hoff enthalpies, they were chosen for comparison with the calorimetric enthalpies, ΔH_s, in determining the cooperativity of the unfolding transition of β-lactoglobulin in solution. ΔH_l is not thermodynamically correct because it was determined with a straight baseline instead of a sigmoidal baseline.

Values of the cooperativity are given in Table 1 and a least-squares fit of the cooperativities to the transition temperature yields

$$\Delta H_s/\Delta H_{vf} = (0.974 \pm 0.007) + (0.003 \pm 0.003)(T_m - 358.0) \quad (6)$$

which does not show a temperature dependence. This is in contrast with the cooperativities of the ribonuclease a [1] and lysozyme [2] transitions which show a slight increase with increase in T_m. The cooperativity of 0.974 ± 0.007 is about 8% less than that observed by Privalov and Khechinashvili [9] for the globular proteins. A cooperativity of less than unity implies slight aggregation of the native protein [11] which was also implied by the transition temperature dependence on concentration at pH 2.6. However, the near equality of the cooperativity to unity shows that the unfolding transition of β-lactoglobulin is two state with a stoichiometry of unity.

Error analysis of the enthalpy data

To determine the possible sources of error in the enthalpy determinations, the T_m dependence of the more precise van't Hoff enthalpies, ΔH_{vf}, and the calorimetric enthalpies with the sigmoidal baseline, ΔH_s, were examined in more detail. The transition temperature dependence of ΔH_{vf} and ΔH_s were determined for subsets of the data, consisting of a lower buffer concentration of 0.1 M, a faster scan rate of 60 K h^{-1}, two different concentration levels, the four different sources of β-lactoglobulin and the three different DSC cells used in the measurements. The results of the least-squares fits of the data in the subsets are presented in Table 3 for the ΔH_{vf} determinations and in Table 4 for the ΔH_s determinations. Since the slopes of the fits for the subsets were the same as for the complete set, the intercepts of the fits were compared to determine how well each of the subsets followed the same temperature dependence as the complete set. The comparisons of ΔH_0 in

TABLE 3

Results of the linear fit $\Delta H_{vf} = \Delta H_{vf,0} + \Delta C'_{p,vf}(T_m - 358.0)$ for β-lactoglobulin in HCl–glycine-buffered solutions under various experimental conditions

Data set	$\Delta H_{vf,0}$ (kJ mol^{-1})	$\Delta C'_{p,vf}$ (kJ K^{-1} mol^{-1})	σ (kJ mol^{-1})	No. of data points	Max. σ(%)	%Deviation of $\Delta H_{vf,0}$
All data	438.6 ± 2.8	1.8 ± 1.0	26.0	85	6	
0.1 M HCl–glycine buffer	425.1 ± 8.0	−0.05 ± 3.5	19.7	7	5	3
Scan rate of 60 K h^{-1}	437.9 ± 4.5	3.8 ± 1.5	13.3	10	3	0
High conc. ≥ 0.352 mM	423.9 ± 4.9	2.2 ± 2.0	19.5	22	5	3
Low conc. ≤ 0.283 mM	445.4 ± 4.6	1.2 ± 1.6	26.5	33	6	2
Cell 1, all sources	429.5 ± 4.4	1.8 ± 1.6	25.9	35	6	2
Cell 2, all sources	441.7 ± 2.5	1.6 ± 2.0	25.6	23	6	0
Cell 3, all sources	447.5 ± 4.7	5.9 ± 1.6	24.3	27	5	2
Sample from source c	444.7 ± 3.6	3.9 ± 1.3	18.5	27	4	1
Sample from source d	457.3 ± 5.3	3.5 ± 1.7	23.7	20	5	4
Sample from source e	425.4 ± 3.2	−0.7 ± 1.5	18.0	22	4	3
Sample from source f	396.7 ± 12	2.8 ± 4.0	31.6	8	8	10
Pure β-lactoglobulin A	409.9 ± 7.8	0.2 ± 3.5	15.1	4	–	–
Pure β-lactoglobulin B	438.6 ± 14	−8.7 ± 5.0	27			

terms of the percentage difference between the intercept of the complete set and of the different subsets are also presented in Tables 3 and 4.

The DSC subset of data performed at the faster scan rate of 60 K h^{-1}

TABLE 4

Results of the linear fit $\Delta H_s = \Delta H_{s,0} + \Delta C'_{p,s}(T_m - 358.0)$ for β-lactoglobulin in HCl–glycine-buffered solutions under various experimental conditions

Data set	$\Delta H_{s,0}$ (kJ mol^{-1})	$\Delta C'_{p,s}$ (kJ K^{-1} mol^{-1})	σ (kJ mol^{-1})	No. of data points	Max. σ(%)	%Deviation of $\Delta H_{vf,0}$
All data	427.1 ± 4.2	0.5 ± 1.5	38.6	85		
0.1 M HCl–glycine buffer	409.9 ± 16	−6.2 ± 6.6	37.2	7	10	4
Scan rate of 60 K h^{-1}	437.9 ± 4.5	3.8 ± 1.5	13.3	10	3	3
High conc. ≥ 0.352 mM	414.2 ± 9.0	−4.1 ± 3.6	35.5	22	9	3
Low conc. ≤ 0.283 mM	440.1 ± 7.0	0.1 ± 2.4	40.2	33	9	3
Cell 1, all sources	421.5 ± 6.2	0.1 ± 2.2	36.2	35	9	1
Cell 2, all sources	429.9 ± 9.0	0.7 ± 3.3	42.2	23	10	1
Cell 3, all sources	432.0 ± 7.9	1.1 ± 2.7	40.5	27	9	1
Sample from source c	438.2 ± 7.1	2.6 ± 2.6	36.2	27	8	3
Sample from source d	443.9 ± 7.4	2.2 ± 2.3	33.1	20	8	4
Sample from source e	408.8 ± 8.3	−3.4 ± 3.2	38.8	22	10	5
Sample from source f	396.7 ± 12	2.8 ± 4.0	31.6	8	8	8
Pure β-lactoglobulin A	410.8 ± 6.9	3.4 ± 3.1	13.4	4		
Pure β-lactoglobulin B	419.6 ± 20	−12.4 ± 7.2	38.9	4		

yielded a linear fit for ΔH_{vf} essentially the same as for all the data. Linear fits of ΔH_s to T_m at the faster scan rate yielded an intercept 3% higher than with the fit for the complete set. The least-squares fits at the faster scan rate are also better, yielded a maximum σ of 3% whereas the maximum σ was 9% for the whole set. (Maximum values of σ were calculated by dividing the standard deviation of the fit by the value of the enthalpy at the lowest transition temperature.) Apparently the enthalpy values and fit are independent of scan rate and, interestingly, the ΔH_s fit is closer to the ΔH_{vf} fit at the faster scan rate.

DSC measurements on solutions at the lower buffer concentration of 0.1 M HCl–glycine yield linear fits of ΔH_{vf} and ΔH_s to T_m with intercepts respectively 3% and 4% lower than the corresponding intercepts of the complete data set fits. Again, these percentages are less than the maximum σ of the fits as shown in Tables 3 and 4. It appears that the enthalpy determinations, ΔH_{vf} and ΔH_s, are independent of buffer concentration from 0.1 to 0.2 M HCl–glycine.

The intercepts describing the linear dependence of ΔH_{vf} and ΔH_s on T_m for the two concentration subsets, ≥ 0.352 mM and ≤ 0.283 mM, agree within 3% with the intercept of the fit from the complete set; the high concentrations yield a lower intercept while the low concentrations yield a higher intercept. The maximum standard deviation of the ΔH_{vf} fits is $\leq 6\%$ while for the ΔH_s fits it is $\leq 9\%$. Since the difference between the intercepts of the fit of the concentration subsets and the complete set is well within the maximum standard deviation of the fit, the linear fits of ΔH_s and ΔH_{vf} to T_m are independent of concentration. The lower value of the intercept, ΔH_0, in the higher concentration range could reflect a contribution of a positive ΔH of association for aggregation of the β-lactoglobulin at the higher concentration. As observed by the slight increase in T_m with concentration at pH 2.6, some of the protein could exist as aggregates in the native state and unfold into monomers in the denatured state.

Comparison of the intercepts for the subsets of data from cells 1, 2, and 3, all of which were calibrated separately, yield deviations of 0–2% from those of the intercepts of the complete sets in Tables 3 and 4. These deviations are less than the maximum standard deviation of the fits in each case. It should be noted that data from each of the three cells used in the DSC measurements yield the same results.

The results of the linear fits with the different source subsets in Table 3 (ΔH_{vf}) show that the intercepts of the fit from sources c, d, and e deviate less than 4% from the intercept of the fit for the complete set. Similarly, the intercepts for the ΔH_s fits of sources c, d, and e deviate less than 5% from the intercept of the complete data set. However, the fewer data points from source f exhibit intercepts with a much larger deviation of 10% and 8% from the intercepts of the complete set. The enthalpy vs. transition temperature fits of the data from this source also yield a maximum standard deviation of

8%, almost twice that of the fits from the other sources, which may reflect the use of fewer data points for the fits. Also, the samples from source f contain the largest amount of β-lactoglobulin A ([A]/[B] = 1.5). However, there does not seem to be any correlation between the standard deviation of the fit and the amount of A for the other three sources as [A]/[B] varies from 1 (source c) to 1.3 (source d). From the standpoint of the less than 5% disagreement between the intercepts from sources c, d, and e, it appears that the enthalpy data are independent of the source of β-lactoglobulin.

The results of the fits from solutions of the pure components, β-lactoglobulin A and B, are also presented in Tables 3 and 4. The intercepts and slopes of the fits of the pure components are within the range of the corresponding values for the fits from sources c–f. Apparently, the unfolding transitions of the pure components exhibit the same enthalpy values and dependences on T_m as their mixtures over the pH range 2.3–3.5

The linear least-squares fits of the enthalpies to T_m yield a maximum standard deviation of 9% for the ΔH_s fits and 6% for the ΔH_{vf}, which are worse than the maximum standard deviations of the fits for lysozyme (5% and 4%, respectively). Part of the error could result from operation of the DSC near its maximum operating temperature, a region where the sensing elements may not respond as precisely as for the lysozyme measurements below 349 K. In fact, the DSC response exhibits a high degree of curvature near the maximum operating temperature which could introduce additional error in the extrapolation of the pre- and post-transitional baselines to the midpoint of the transition peak. The worse fit with the ΔH_l enthalpies, calculated with a straight baseline in Fig. 3, is the result of neglecting this curvature by using a straight baseline. Additional sources of error in the determination of ΔH_{vf} include a 1% uncertainty in the concentration determinations and a 1% uncertainty in weighing the sample cells. There is also an uncertainty in choosing the correct temperature limits for fitting the transition peak to a two-state model.

CONCLUSIONS

The transition enthalpies for β-lactoglobulin in 0.2 M HCl–glycine-buffered solutions can be determined with sufficient accuracy and reproducibility for the evaluation of DSC performance in the 353–362 K temperature range. DSC scans of dialyzed solutions of β-lactoglobulin in HCl—glycine buffer yield transition enthalpies which are independent of the source of the β-lactoglobulin, of the buffer concentration from 0.1 to 0.2 M, and of the scan rate. Furthermore, the transitions are two state, and thus amenable to analysis by the two-state transition model. As the ΔH_{vf} enthalpies were determined with a temperature correction, they are the most accurately determined van't Hoff enthalpies. Because the ΔH_s enthalpies

were determined with the sigmoidal baseline, they are the correct calorimetric enthalpies. The ΔH_{vf} enthalpies of the unfolding transition of 0.03–0.45 mM β-lactoglobulin in 0.2 M HCl–glycine-buffered solutions exhibit the following transition temperature dependence over the pH range 2.3–3.5:

$$\Delta H_{vf}(\text{kJ mol}^{-1}) = (438.6 \pm 2.8) + (1.8 \pm 1.0)(T_m - 358.0) \quad (7)$$

while the ΔH_s enthalpies exhibit the dependence

$$\Delta H_s(\text{kJ mol}^{-1}) = (427.1 \pm 4.2) + (0.5 \pm 1.5)(T_m - 358.0) \quad (8)$$

ACKNOWLEDGEMENT

The author wishes to thank William H. Kirchhoff of the Department of Energy for the use of his Exam Program in the analysis of the DSC thermal scans. This program is available upon request by writing to the author or to William H. Kirchhoff, c/o Chemical Thermodynamics Division, National Institute of Standards and Technology.

REFERENCES

1 F.P. Schwarz and W.H. Kirchhoff, Thermochim. Acta, 128 (1988) 267.
2 F.P. Schwarz, Thermochim. Acta, 147 (1989) 71.
3 J.N. De Witt and G.A.M. Swinkels, Biochim. Biophys. Acta, 624 (1980) 40.
4 K.A. Piez, E.W. Davie, J.E. Folk, and J.A. Gladner, J. Biol. Chem., 236 (1961) 2912.
5 N.K.D. Kella and J.E. Kinsella, Biochem. J., 255 (1988) 113.
6 L.L.Y. Lee and J.C. Lee, Biochemistry, 26 (1987) 7813.
7 M. Paulsson, P.O. Hegg and H.B. Castberg, Thermochim. Acta, 95 (1985) 435.
8 R. Townend, R.J. Winterbottom and S.N. Timasheff, J. Am. Chem. Soc., 82 (1960) 3161.
9 P.L. Privalov and N.N. Khechinashvili, J. Mol. Biol., 86 (1974) 665.
10 S.E. Zale and A.M. Klibanov, Biochemistry, 25 (1986) 5432.
11 T.Y. Tsong, R.P. Hearn, D.P. Wrathall and J.M. Sturtevant, Biochemistry, 9 (1970) 2666.
12 P.L. Privalov, Adv. Protein Chem., 33 (1979) 167.