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Thermodynamic parameters for beta-lactoglobulin dissociation over a broad temperature range at pH 2.6 and 7.0

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Abstract

Thermodynamic parameters were determined for the dissociation of beta-lactoglobulin(β-Lg) at temperatures from −15 to 85°C. The effect of temperature on K_d (equilibrium constant for dimer ⇌ monomer dissociation) was described by a second-order Van't Hoff equation ($\ln K_d = AT^{-2} + BT^{-1} + C$) or Gibbs–Helmholtz equation. The Gibbs free energy (ΔG), enthalpy (ΔH), entropy (ΔS) and thermal capacity (ΔC_p) for β-Lg dissociation were evaluated. At 25°C standard temperature thermodynamic parameters were ΔG^0=24.8 (±0.35) kJ mol^{-1}, ΔH^0=57 (±13) kJ mol^{-1}, ΔS^0=92 (±30) J mol^{-1} K^{-1} and ΔC_p=2383 J mol^{-1} K^{-1} at pH 2.6. For β-Lg dissociation at pH 7, ΔG^0=28.6 (±2.7) kJ mol^{-1}, ΔH^0=107.5 (±6.3) kJ mol^{-1}, ΔS^0=265.7 (±39) J mol^{-1} K^{-1} and ΔC_p=2383 J mol^{-1} K^{-1}. Simulated temperature–dissociation profiles of β-Lg show that the fraction of dissociated protein increases with increasing temperature, decreasing pH and with decreasing protein concentration.

Keywords: Beta-lactoglobulin; Dissociation; Protein stability; Thermodynamics

1. Introduction

The effect of the quaternary (4°) structure on the thermal stability of proteins having more than one subunit is not well-understood [1]. However, quantitative solvent denaturation studies involving such multi-subunit proteins are gaining momentum [2]. The urea unfolding stability of beta-lactoglobulin (β-Lg) was underestimated over the past 30 years. Denaturation models suited for multi-subunit proteins have only just been applied to this dimeric protein. Our studies show that 32 and 43% of the conformational stability of β-Lg arises from subunit association at pH 2.6 and 7, respectively [3]. The effect of 4° structure on β-Lg thermal stability has not been assessed quantitatively. Increasing the accuracy of protein stability measurements is important for scientific reasons. The subject has practical significance in attempts to develop protein stability-function correlations (cf. [4] and references cited therein).

Understanding of β-Lg dissociation stability is a necessary step to assessing its overall thermal stability. Dissociation is the first stage in the thermal denaturation of β-Lg and other multi-subunit proteins. Thermodynamic parameters are not available for the dissociation of β-Lg over a broad temperature range. In this paper, the two foremost techniques for treating thermal denaturation results for simple proteins [5,6] were modified and applied to β-Lg. Possessing two identical subunits, β-Lg is an excellent model for studies of protein dissociation [7–12].

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Values of the Gibbs free energy (ΔG), enthalpy (ΔH), entropy (ΔS) and thermal capacity (ΔC_p) change for the dissociation of β-Lg at pH 2.6 or 7 at temperatures from −15 to 85°C are reported. Simulated temperature–dissociation profiles based on these thermodynamic parameters reveal how the dissociation β-Lg is affected by protein concentration, solvent temperature and pH. This paper provides a simple protocol for analysing thermal denaturation results for dimeric proteins.

2. Materials and methods

2.1. Theory

Data usually collected at denaturation temperatures need to be extrapolated (or interpolated) to a standard temperature of 25°C. The Van't Hoff or Gibbs–Helmholtz equations [5,6] give satisfactory results for single subunit proteins. For small globular proteins having one subunit, thermal denaturation may be described by

$$N \rightleftharpoons U \tag{1}$$

where N and U are the native and partially unfolded states, respectively. For a two-state, reversible, equilibrium process the denaturation equilibrium constant (K) and the fraction of protein denatured (F_{den}) are described by Eqs. (2), (3a) and (3b), respectively.

$$K = \frac{[U]}{[N]} \tag{2}$$

$$F_{\text{den}} = \frac{[U]}{[N]+[U]} = \frac{K}{1+K} \tag{3a}$$

$$K = \frac{F_{\text{den}}}{1-F_{\text{den}}} \tag{3b}$$

It is difficult to measure $[U]$, F_{den} or K accurately at low temperatures because of the high N:U ratio under such circumstances. Increasing the sample temperature raises the concentration of the U-state. Therefore, K (or F_{den}) is usually determined with greater accuracy at high temperatures. The Van't Hoff or Gibbs–Helmholtz equations are then used to interpolate data from high to low temperatures.

Van't Hoff equation: The temperature-dependence of ln K is described by a second order equation,

$$\ln K = \frac{A}{T^2} + \frac{B}{T} + C \tag{4}$$

where T is the temperature (in Kelvin). The constants A, B and C are determined by nonlinear regression (NLR). $\mathrm{d}(\ln K)/\mathrm{d}(1/T) = -\Delta H/R$ and furthermore $\mathrm{d}(\Delta H)/\mathrm{d}T = \Delta C_p$ therefore

$$\Delta H = -R\left(\frac{2A}{T} + B\right) \tag{5}$$

$$\Delta C_p = \frac{-2RA}{T^2} \tag{6}$$

Other thermodynamic relations of note are $\Delta G = -RT \ln K$ and $\Delta S = (\Delta H - \Delta G)/T$. After fitting experimental data to Eq. (4) we can (a) extrapolate values of ln K outside the experimental temperature range and (b) determine thermodynamic parameters for the process of interest [5].

Gibbs–Helmholtz equation: ΔG varies with temperature according to Eq. (7), where T_{m} is the temperature required to denature 50% of protein molecules [6].

$$\Delta G = \Delta H_{\text{m}}\left(1 - \frac{T}{T_{\text{m}}}\right) - \Delta C_p\left[(T_{\text{m}} - T) - \ln\left(\frac{T}{T_{\text{m}}}\right)\right] \tag{7}$$

The above is a consequence of the following well known relations

$$\Delta G = \Delta H - T\,\Delta S \tag{8}$$

$$\Delta H = \Delta H_{\text{m}} + \Delta C_p(T - T_{\text{m}}) \tag{9a}$$

$$\Delta S = \Delta S_{\text{m}} + \Delta C_p \ln\left(\frac{T}{T_{\text{m}}}\right) \tag{9b}$$

where, ΔH_{m} and ΔS_{m} are the enthalpy and entropy change for $T = T_{\text{m}}$, respectively. ΔG vs. temperature data is fitted to Eq. (7) by NLR.

A less demanding computational use of Eq. (7) is as follows. First, ΔH_{m} and T_{m} must be found using a first-order Van't Hoff equation

$$\ln K = \frac{-\Delta H}{RT} + Z \tag{10}$$

For data collected near T_{m}, plotting ln K versus $1/T$ yields a straight-line graph with a slope ($= -\Delta H/R$) which is independent of temperature. The "Y=0"

intercept is Z. When $T=T_m$ then $\Delta H=\Delta H_m$. In addition, at the temperature for 50% denaturation, ln $K=0$ and $T_m=\Delta H_m/(RZ)$. In other words, T_m=slope/intercept. Furthermore,

$$Z = \frac{\Delta H_m}{RT_m} \tag{11}$$

Perform this study at two pH values or at different urea concentrations and two estimates of ΔH_m and T_m will be found from which ΔC_p may be determined [13]. $\Delta C_p=\Delta\Delta H_m/\Delta\Delta T_m$. ΔC_p can also be estimated from the protein molecular weight or from changes in the solvent accessible surface area (SSA) produced by denaturation [14]. Next, values of H_m, T_m, and ΔC_p are inserted into Eq. (7) to produce a ΔG–temperature curve [6]. Other thermodynamic parameters are evaluated using H_m, T_m, and ΔC_p as indicated in Eqs. (9a) and (9b).

2.2. Methods

K_d–temperature results used for this investigation were obtained from three sources. Visser et al. [8] studied the dissociation of a mixture of β-Lg genetic variants A and B at 5–35°C (pH 2.6) by ultracentrifugation. Joss and Ralston [7] determined the association of β-Lg B at pH 2.6 at 5–30°C also by ultracentrifugation. Both studies involved glycine–HCl buffer (0.16 M) as solvent. The protein concentration was between 0.7 and 1.5 mg ml^{-1}. Aymard et al. [9] monitored the dissociation of β-Lg by static and dynamic light scattering from 5 to 76°C at pH 7.0. The solvent was ammonium acetate buffer (0.1 M). Values for K_d from [7–9] agree with a variety of reports by others at ~20–25°C [10–12]. In summary, the data used in this investigation are representative of the behaviour of β-Lg at pH 2.6 and 7 (ionic strength of 0.1–0.16).

Data treatment methods described for single subunit proteins were modified and applied to β-Lg. In the case of the second-order Van't Hoff relation, values for K_d at 5–35°C [7,8] were fitted to Eq. (4) and the constants A, B, and C estimated by NLR. Additional K_d values were determined at temperatures between −15 and 85°C using Eq. (4) and known equation parameters (A, B and C). Next, the fraction of dissociated protein (α) was determined using Eq. (14). The protein concentration term in Eq. (14) was then altered over the range of 0.05–100 mg ml^{-1}. ΔC_p and other thermodynamic parameters were determined from known values of A, B, and C (cf. Eqs. (5) and (6)).

The Gibbs–Helmholtz equation was applied to K_d values from 5 to 76°C [9]. First, ln K_d was plotted versus 1/T (cf. Eq. (10)). The constants ΔH_m and T_m were determined from the slope and intercept (cf. Eqs. (15) and (16)). ΔC_p was taken from results obtained in the preceding paragraph; this was possible because ΔC_p is not believed to be pH dependent. From values for ΔH_m, T_m and ΔC_p then ΔG was estimated over a wide temperature range using Eq. (7). The initial K_d vs. temperature data was also analysed using Eq. (4). All calculations were performed using EXCEL™ spreadsheet in a Microsoft-Windows 97 environment.

3. Results and discussion

The thermal denaturation process for β-Lg can be described by Eq. (12). The protein dimer (D_n) dissociates to form native monomers (N). These unfold forming the U-state.

$$D_n \rightleftharpoons nN \rightleftharpoons nU \tag{12}$$

The focus of this paper is on the $D_n \rightleftharpoons nN$ reaction. For a protein having n-subunits, the equilibrium constant for protein dissociation K_d (=$[N]^n/[D_n]$) may be expressed by

$$K_d = \frac{n^2 P^{(n-1)} \alpha^n}{1-\alpha} \tag{13}$$

where α is the fraction of protein dissociated and P the total concentration of protein calculated using the formula for D_n. For a dimeric protein, n=2. Re-writing Eq. (13) as a quadratic ($4\alpha P^2+\alpha K_d-K_d=0$) and solving for α gives the relation below for the positive root.

$$\alpha = \frac{-K_d + (K_d^2 + 16PK_d)^{1/2}}{8P} \tag{14}$$

3.1. Effect of temperature and protein concentration on the dissociation of β-Lg

A graph of ln K_d versus 1/T (Eq. (4)) is shown in Fig. 1. The equation for the continuous line is (Fig. 1a) $\ln K_d=1.6680\times10^7(T^{-2})-1.1961\times10^5(T^{-1})+203.58$.

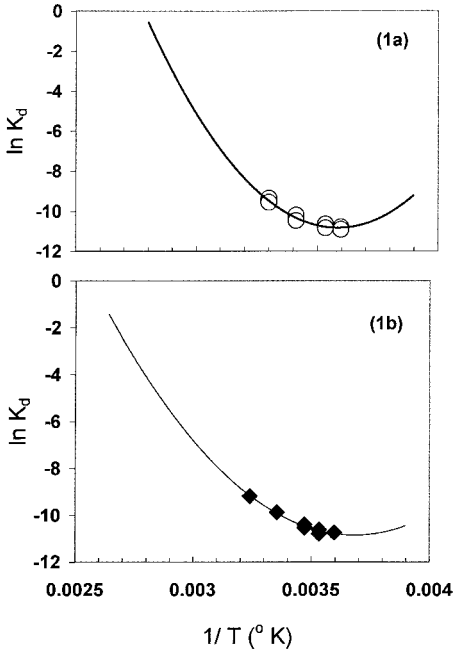

Fig. 1. Van't Hoff plot for the dissociation of β-Lg at pH 2.6. (a) Results for β-Lg genetic variants A and B at pH 2.6 (points show data from [7]); (b) Results for a mixture of β-Lg genetic variants A and B at pH 2.6 (points show data from [8]). The calculated (continuous) lines are described by $\ln K_d = 1.6680\times10^7\ (T^{-2}) - 1.1961\times10^5\ (T^{-1}) + 203.58$ (1a) and $\ln K_d = 8.6952\times10^7\ (T^{-2}) - 6.4034\times10^4\ (T^{-1}) + 107.53$ (1b).

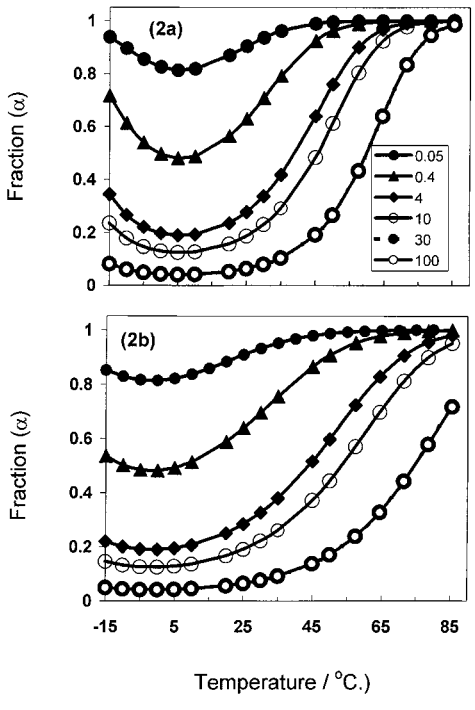

Fig. 2. Effect of temperature and β-Lg concentration on the fraction of protein dissociated (α) at pH 2.6. The boxed legend shows β-Lg concentration (mg ml^{-1}). (a) and (b) were calculated from data in Fig. 1a and b, respectively (see text for details).

From Fig. 1b, $\ln K_d = 8.6952\times10^7\ (T^{-2}) - 6.4034\times10^4\ (T^{-1}) + 107.53$. Fig. 1a shows the dissociation behaviour of β-Lg genetic variant B [7]. Fig. 1b is the corresponding result for a mixture β-Lg genetic variants A and B [8]. The high regression coefficients (r=0.9858 and 0.9930, respectively) show good agreement between experimental and calculated results.

Simulated temperature–dissociation profiles for β-Lg B and a mixture of β-Lg A and B are shown in Fig. 2a and b. The profiles are based on results from Fig. 1. The concentration of β-Lg examined, though arbitrary (0.05–100 mg ml^{-1}), covers values likely to be encountered in most situations. The amount of the β-Lg in bovine milk whey is about 2–4 mg ml^{-1}. Thermal unfolding studies usually involve low protein concentrations ($\leq$4 mg ml^{-1}) in order to avoid aggregation. Investigations of thermal gelation involve β-Lg concentrations of $\geq$50 mg ml^{-1} (>5% w/w protein).

The characteristics of the β-Lg dissociation reaction at pH 2.6 can be summarised as follows (Fig. 2): (1) β-Lg exists as the monomer (>70% dissociation) at low protein concentrations ($\leq$0.4 mg ml^{-1}); (2) at levels exceeding 4 mg ml^{-1} the predominant form is a dimer; (3) the *apparent* temperature for 50% dissociation ($T_{m(app)}$) increases with protein concentration; (4) no value can be assigned to $T_{m(app)}$ at low protein concentrations because α>0.5 at all temperatures between −15 and 85°C; (5) with a β-Lg concentration of 4 mg ml^{-1}, α=0.5 at 42°C and at −20°C, i.e., two $T_{m(app)}$ values are evident.

Fig. 3a shows temperature–dissociation profiles based on the Gibbs–Helmholtz equation. The initial data for these investigations were for a mixture of β-Lg A and B monitored at temperatures between 5 and 76°C at pH 7.0 [9]. First, values for T_m, ΔH_m were determined using Eq. (10). A straight-line graph was obtained by plotting ln K vs. $1/T$ (results not shown). The slope of this graph is $-\Delta H_m/R$. For a dimeric

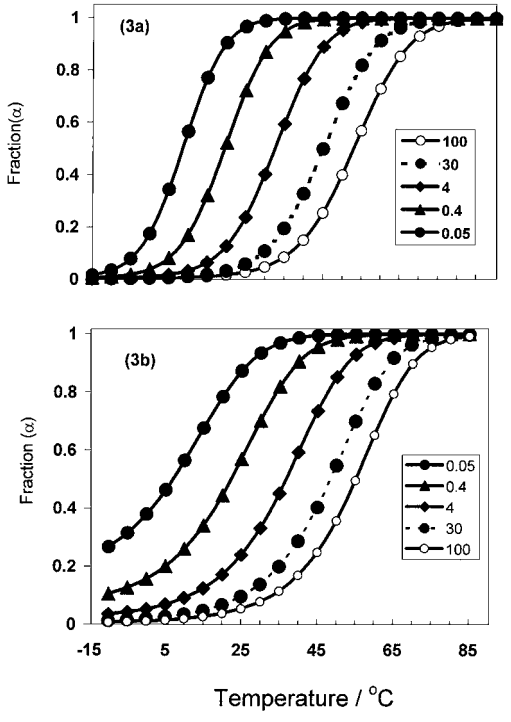

Fig. 3. Effect of temperature and β-Lg concentration on the fraction dissociated (α) at pH 7.0. The boxed legend shows β-Lg concentrations (mg ml^{-1}). Profiles were determined with the aid of the extended Van't Hoff equation (Eq. (3b)) or Gibbs–Helmholtz function (Eq. (3a)).

protein the "Y=0" intercept (Z^*) varies with protein concentration [1].

$$Z^* = \frac{\Delta H_m}{RT_m} + (n-1)\ln P \quad (15)$$

Dividing the slope by the intercept gives an apparent melting temperature $T_{m(app)}$ which varies with protein concentration. T_m was determined from

$$T_m = \frac{\Delta H_m/R}{Z^* - (n-1)\ln P} \quad (16)$$

ΔH_m, T_m and ΔC_p estimates determined in this manner were inserted into Eq. (7). From the resulting Gibbs–Helmholtz equation we found ΔG (and $\ln K_d$) values from −15 to 85°C. The fraction of protein dissociated was then determined (cf. Eq. (14)) as before. For comparison, the second-order Van't Hoff equation was used to analyse the same pH 7 data [9]. The equation of the line was $\ln K_d = 9.7175\times10^6\ (T^{-2}) - 7.8804\times10^4\ (T^{-1}) + 144.70$ (r=0.9930). Again, values for α were determined from Eq. (14) as described previously. Temperature–dissociation profiles modelled with the aide of the Gibbs–Helmholtz analysis (Fig. 3a) and Van't Hoff analysis (Fig. 3b) are similar except at low β-Lg concentrations.

In contrast, with results from pH 2.6, there were no low-temperature dissociation transitions at pH 7. At each protein concentration, we found a single $T_{m(app)}$ value. This increased from 7 to 50°C for β-Lg concentration of 0.05 and 100 mg ml^{-1}. Comparing Fig. 2 with Fig. 3 shows that β-Lg is more weakly associated at pH 2.6 compared to pH 7. This is attributed to greater non-specific charge–charge repulsion bet ween the β-Lg subunits at low pH [10–12]. However, differences in dimerisation tendency at pH 2.6 and 7 disappear at high (>4 mg ml^{-1}) β-Lg concentrations.

3.2. Thermodynamic parameters for β-Lg dissociation

In the majority of studies, thermodynamic parameters for β-Lg dissociation were treated as independent of temperature [8–12]. Curved Van't Hoff graphs for β-Lg dissociation were thought to show "a break" and two limiting ΔH values [8]. Joss and Ralston [7] reported a curved Van't Hoff graph for β-Lg subunit association. They explained such results in terms of the temperature-dependence of ΔH between 5 and 30°C. A significant thermal capacity difference between β-Lg dimer and monomer was postulated [7]. However, no attempts were made to evaluate ΔC_p.

The effect of temperature on ΔH, ΔS and ΔG values for β-Lg dissociation are shown in Fig. 4. The profiles were calculated using K_d values for β-Lg B at pH 2.6 [7] in conjunction with the second-order Van't Hoff equation. Similar profiles were obtained from the analysis of data from [8,9] (data not shown). Results of ln K_d vs. l/T were translated into a ΔG–temperature profile (Fig. 4b). The latter graph shows that β-Lg dimer is more stable at pH 7 compared to pH 2.6 as expected. The same ln K_d vs. l/T data were also fitted with NLR equation parameters (A, B, C). From the equation parameters, ΔH and ΔS values were established over a wide range of temperatures as explained above (cf. Eqs. (4)–(6) and associated text).

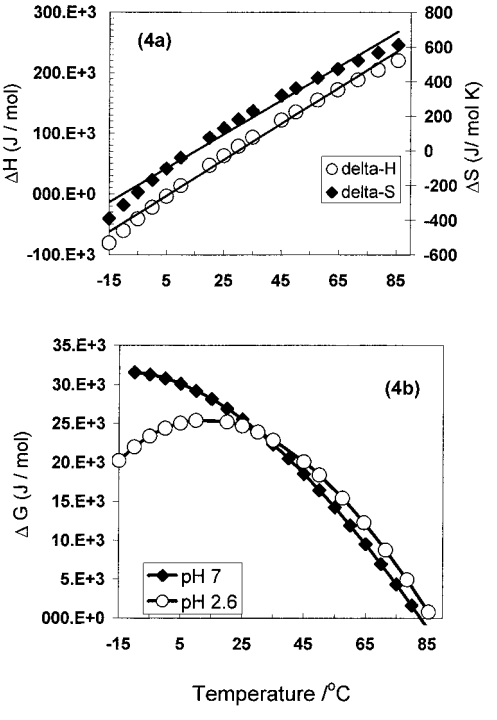

Fig. 4. Effect of temperature on thermodynamic parameters for β-Lg dissociation. (a) Values at pH 2.6 (cf. Fig. la); (b) Gibbs free energy change for dissociation at pH 2.6 or 7 as a function of temperature. All results were determined with second-order Van't Hoff equation.

3.3. On the differences between the Van't Hoff and Gibbs–Helmholtz treatments

To check the accuracy of ΔH and ΔS values (Fig. 4a), we attempted to re-evaluate ΔG^0 (from $\Delta H^0 - T\Delta S^0$). This requires that ΔH and ΔS values are found at 25°C. Consider only values of ΔH for a moment. One can proceed by calculating ΔH^0 from Eq. (5), which is the function used to generate Fig. 4a. The alternative approach based on the Gibbs–Helmholtz treatment, is to determine ΔH^0 using Eqs. (9a) and (9b). According to this relation, ΔH increases linearly with temperature. The almost trivial exercise of calculating ΔG^0 (from $\Delta H^0 - T\Delta S^0$) offered an interesting opportunity to compare results for the Van't Hoff and Gibbs–Helmholtz treatments closely.

First, using Gibbs–Helmholtz formalisms the following straight lines give apparently good fits to the data in Fig. 4a.

$$\Delta H \ (\text{pH } 2.6) = 2974.4 \ (T) - 829.3 \times 10^3 \quad (r = 0.9950) \tag{17a}$$

$$\Delta H \ (\text{pH } 7) = 1702.4 \ (T) - 398.4 \times 10^3 \quad (r = 0.9965) \tag{17b}$$

$$\Delta S \ (\text{pH } 2.6) = 9.8221 \ (T) - 2831.7 \quad (r = 0.9903) \tag{17c}$$

$$\Delta S \ (\text{pH } 7) = 5.5694 \ (T) - 1386.5 \quad (r = 0.9922) \tag{17d}$$

ΔH^0 and ΔS^0 values were then obtained with Eqs. (17a)-(17d) for T=298K. Thereafter, we determined ΔG^0 (from $\Delta H^0 - \mathrm{T}\Delta S^0$) as 28.18 kJ mol^{-1} at pH 2.6 and 27.6 kJ mol^{-1} at pH 7. Interestingly, such results are unsatisfactory. The value for ΔG^0 at pH 2.6 was 4–6 kJ mol^{-1} greater than literature ΔG^0 values [7,11–13]. Moreover, Van't Hoff ΔG–temperature profiles in Fig. 4b show clearly that protein stability was greater at pH 7 compared to pH 2.6 as reported in the literature.

The error in the ΔG^0 estimate at pH 2.6 was symptomatic of some hitherto unrecognised feature of Eqs. (17a)-(17d) and the Gibbs–Helmholtz analysis. The origin of the error was traced to the following possible source. Both Eqs. (5) and (9a) or (17a)-(17b) describe the temperature-dependence of ΔH. Eq. (5) from the Van't Hoff treatment allows ΔC_p to change with temperature. Eq. (9a) or (17a)-(17b) from the Gibbs–Helmholtz treatment assume that $\mathrm{d}(\Delta C_p)/\mathrm{d}T$=0. In using Eqs. (17a)-(17d) to estimate ΔH^0 and ΔS^0, the condition $\mathrm{d}(\Delta C_p)/\mathrm{d}T$=0 was inadvertently imposed. However, close scrutiny of the lines in Fig. 4a show these are curved. Fitting a straight-line function to the data (cf. Gibbs–Helmholtz) leads to errors in ΔH^0 and ΔS^0 which carryover to the estimate of ΔG^0.

Relations examined in this paper place different emphasis on ΔC_p. The least sophisticated analysis, involving the first-order Van't Hoff equation (Eq. (10)), assumes that ΔC_p=0. Next comes the Gibbs–Helmholtz function (Eqs. (7)-(9b), Eqs. (17a)-(17d)) from which it can be seen that $\mathrm{d}(\Delta C_p)/\mathrm{d}T$=0. Finally, the second-order Van't Hoff equation (cf. Eqs. (4)–(6)) allows for changes in ΔC_p with temperature. To consider even stronger dependence of ΔC_p on temperature, Eq. (4) may be expanded. The

resulting third-order Van't Hoff equation is given by

$$\ln K_d = AT^{-3} + BT^{-2} + CT^{-1} + D \quad (18)$$

Moreover, since d(ln K)/d(1/T)=$-\Delta H/R$ and d(ΔH)/dT=ΔC_p we have

$$\Delta H = -R(3AT^{-2} + 2BT^{-1} + C) \quad (19)$$

and

$$\Delta C_p = R(6AT^{-3} + 2BT^{-2}) \quad (20)$$

Fig. 5 shows the temperature–dissociation profile for β-Lg determined with Eq. (18). Previous results based on Eq. (4) are shown for comparison. In both examples, the concentration of β-Lg is the same 4 mg ml^{-1} and the pH is 2.6. The second order Eq. (4) gives two $T_{m(app)}$ values of −20 and 42°C, as described before. By comparison, Eq. (18) also predicts two dissociation transitions. However, the low temperature (<10°C) transition is an association reaction. Both graphs create the impression of two dissociation reactions taking place at low and high temperatures [8]. The curvilinear Van't Hoff plots are a consequence of thermal capacity change for dissociation. To establish the relative accuracy of Eq. (4) or (17), it will be necessary to establish the degree of β-Lg dissociation at −20°C. Fig. 5 reveals that the second and third-order Van't Hoff relations predict opposite dissociation responses at subzero temperatures.

To our knowledge, the present methods for analyses [5,6,15] have not been applied to the dissociation

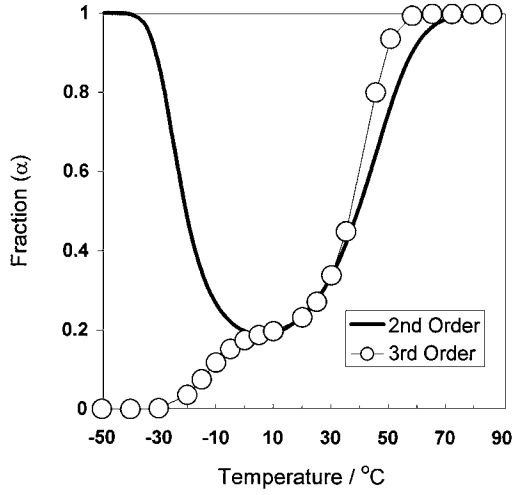

Fig. 5. The effect of a weak (cf. second order plot) or strong ΔC_p temperature-dependence on dissociation profiles for β-Lg.

reaction for β-Lg. These techniques have also not been applied to other oligomeric proteins. Extrapolation methods can be vulnerable to unexpected responses outside of the experimental temperature range. For example, the current treatment takes no account of the thermal unfolding of β-Lg. β-Lg monomers at temperatures above 85 or 75°C at pH 2.6 or 7, respectively [16]. The modelling procedures for generating thermal-dissociation–concentration profiles lead to accurate results. General predictions presented in Figs. 2–4 agree with experimental observations

Table 1
Thermodynamic parameters for the dissociation of β-Lg

Solvent (pH)	Parameters				
	ΔG^0 (kJ mol^{-1})	ΔH^0 (kJ mol^{-1})	ΔS (J mol K^{-1})	ΔC_p (J mol^{-1} K^{-1})	Ref.
2.6	24.56	47.12	75.95	1637	This work[a]
2.6	24.69	63.71	130.9	3131	This work[b]
2.6	25.33	43.47	62.7	–	[7]
2.6	24.66	53.50	97.0	–	[10]
2.6	24.5	77.6	177.9	–	[8]
7	25.60	112.95	293.3	2383[c]	This work[d]
7	29.0	–	–	–	This work[e]
7	31.1	102.0	238	0	[9]

[a] Data taken from [7] using a second-order Van't Hoff equation.
[b] Data taken from [8] using a second-order Van't Hoff equation.
[c] ΔC_p is averaged from two estimates at pH 2.6 (see text for details).
[d] Data taken from [9] using a second-order Van't Hoff equation.
[e] Data taken from [9] using the Gibbs–Helmholtz equation.

[7–12]. Fig. 5 shows more speculative results arising from uncertainty about the temperature derivative for ΔC_p. The initial choice of data [7–9] are sound as are the thermodynamic principles applied. From results at our disposal (Table 1), average thermodynamic parameters for β-Lg dissociation are as follows: At pH 2.6 (25°C) ΔG=24.8 (±0.35) kJ mol^{-1}, ΔH=57 (±13) kJ mol^{-1}, ΔS=92 (±30) J mol^{-1} K^{-1} and ΔC_p= 2383 J mol^{-1} K^{-1}. At pH 7.0 (25°C), ΔG=28.6 (±2.7) kJ mol^{-1}, ΔH=107.5 (±6.3) kJ mol^{-1}, ΔS= 265.7 (±39) J mol^{-1} K^{-1} and ΔC_p=2383 J mol^{-1} K^{-1}. We are currently assessing the thermal denaturation β-Lg and the effect of dissociation on the reaction thermodynamics.

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