Journal of Thermal Analysis, Vol. 37 (1991) 1887-1895

ON THE PARTIAL REVERSIBILITY OF THE β-LACTOGLOBULIN HEAT DENATURATION

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The thermal behavior of β-lactoglobulin (β-lg) disperesed in distilled water ($pH = 3.2$) is studied dy differential scanning calorimetry (DSC) in the temperature range 20°C–120°C and within a concentration region of 3.5% to 24%.

Recently [1] we have determined by DSC the kinetic parameters for the heat-denaturation of β-lg. The effect of the protein concentration and of the thermal treatment on transition temperature (T_{trs}), half-widths of the peak ($\Delta T_{1/2}$), of apparent enthalpy changes ($\Delta_{app}H$) and of Van't Hoff enthalpies ($\Delta_{VH}H$) have been examined for the concentrations of 8.8% and 24%.

In this study we have undertaken complementary experiments for the concentrations 3.5% and 10.8%. The half-widths of the peaks, which depend on the cooperativity of the denaturation process [2], decrease with increasing concentration. The ratio $\Delta_{app}H/\Delta_{VH}H$ tends to 1, with low values of the protein concentration and with high scanning rates. This implies the hypothesis of a reversible step for the denaturation process of β-lg.

Materials

β-lg has been prepared from acid whey concentrates obtained from the Research Dairy Laboratory of Rennes in powdered form with 5% water content [3]. 94% of the proteins are β-lg. The monomer weight is given with 18422 Daltons.The samples were dissolved in distilled water, giving $pH = 3.2$ independent for all concentrations within the limits of 3.5% to 24% (W/W, dry matter basis). The concentrations have been determined from the absorbance at 278 nm, $A^{1\%}_{1cm} = 9.3$.

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Calorimetric measurements

The thermal behavior of the β-lg protein in solution is monitored by differential scanning calorimetry with a Perkin-Elmer DSC7, within the temperature interval from 20° to 120° at several scanning rates.

About 60 mg of protein solution are sealed in stainless steel pans having a total volume of 75 μL. A reference pan with about 60 mg of distilled water has been applied for determination of peak temperature (T_p), enthalpy change accompanying the thermal transition ($\Delta_{app} H$) and half-width of the heat absorption peak ($\Delta T_{1/2}$).

A sealed empty pan was chosen for the determination of half-reaction times ($t_{1/2}$) of the denaturation process. The scan rates have been adjusted or have been altered within given limits according to the necessities of each measurement.

Thermoanalytical curves

Figure 1 shows the endothermic peaks corresponding to several concentrations of β-lg applying a scan rate of 5 deg/min.

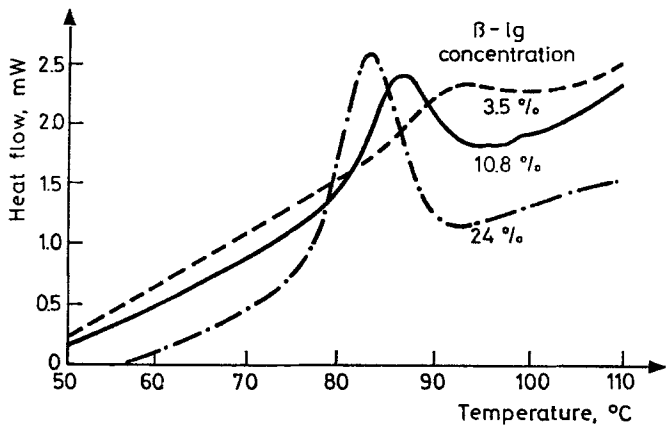

Fig. 1 Effect of the β-lg concentration on the DSC curves (scan rate 5 deg/min)

Peak temperatures and half-width of peaks increase with decreasing concentration of the β-lg.

Figure 2 represents the dependence of the endothermic peak corresponding to 8.8% of β-lg at scanning rates from 2 deg/min to 15 deg/min.

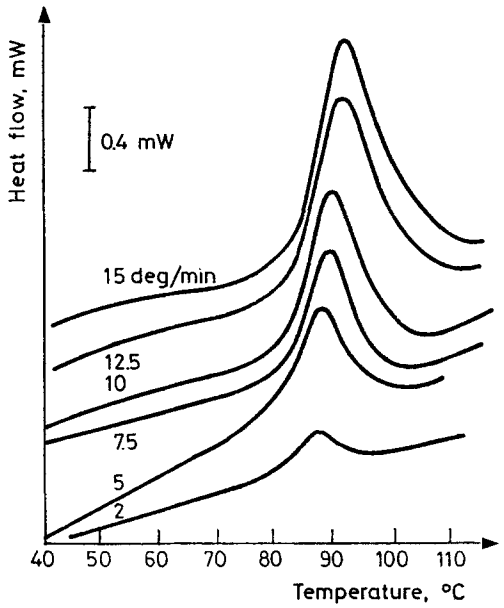

Fig. 2 Effect of the scanning rate on the DSC curves of β-lg solution. (Protein concentration is 8.8%)

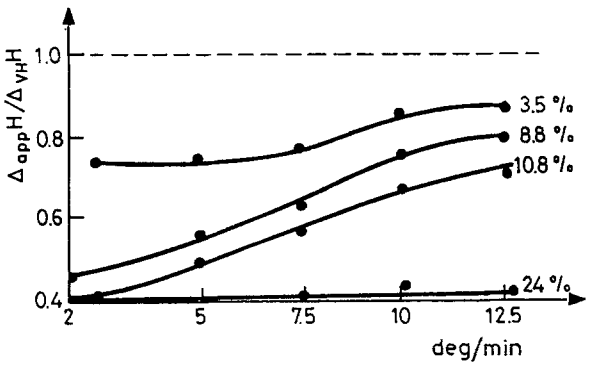

Fig. 3 Effect of the protein concentration on the ratio $\Delta_{app}H/\Delta_{VH}H$ *vs.* the scanning rate

Peak temperature increases with increasing scan rate which is a phenomena typically for kinetic processes.

Figure 3 shows the variation of the ratio of apparent enthalpy change [1] on Van't Hoff enthalpy (Δ_{app} $H/\Delta_{VH}H$) *vs.* the scan rate, for various protein concentrations.

Values of $\Delta T_{1/2}$, T_p, and $\Delta_{app} H/ \Delta_{VH} H$ are collected in Table 1 for different protein concentrations and different scan rates. The transition temperature, T_{trs}, determined by extrapolation at 0 deg/min scan rate decreases with increasing protein concentration. Their values are 88.6°, 85.8°, 85.4° and 83.4° for 3.5%; 8.8%, 10.8% and 24% protein concentration, respectively.

The aggregation phenomenon of β-lg is depending on the concentration of the protein and reaches a maximum at the highest concentration, practically independent from the scan rate. At lower concentrations, the aggregation has only a marginal effect on the thermodynamic parameters. In general, the half width of the heat absorption peaks increases with the scan rate and decreases with the concentration. A similar behavior was observed in our latter study of β-lg denaturation at 8.8% and 24% [1].

These experimental results confirm that the denaturation plays a larger role with decreasing concentration and with increasing scan rate [1]. However, a second heating run of the protein sample did not show repeatable renaturation peak even when the ratio $\Delta_{app} H/\Delta_{VH} H$ tends to its maximum experimental value ($\sim$ 0.9)

Kinetic parameters

In this study the aggregation process is more or less important depending on experimental conditions (concentration, heating time). Therefore, we consider both phenomena as a whole, termed denaturation but implying various degree of aggregation.

The kinetic parameters are determined from two methods:
(i) the one proposed by Kissinger [4], which is based on the variation of peak temperature with the scan rate.
(ii) the method developed by Borchardt and Daniels [5], using a straight or a sigmoidal baseline [6], which takes into account the thermal resistance between heater system and samples.

Figure 4 displays an example of a DSC curve with the following experimental conditions: 3.5%, 60 mg, 7.5 deg/min, from 20° to 120°. In addition a sigmoidal baseline is used. In this case the thermal resistance given by the Perkin-Elmer Software is 25°/W.

The kinetic parameters of the heat denaturation for β-lg are reported in Table 2. There is a general agreement between E_A values calculated with the three methods for all concentrations applied. The half-reaction times decrease with increasing concentration, in accordance with a reaction order of about $n = 2$.

Table 1 Value of peak temperatures (T_p), half-widths of the peaks ($\Delta T_{1/2}$), apparent enthalpy changes (Δ_{app} H), Van't Hoff enthalpy ($\Delta_{VH}H$) at various scanning rates (dT/dt) and β-lg concentrations (sample mass: 60 mg, pH 3.2, heating from 20°C to 120°C)

β-lg con.	dT/dt, °C	$\Delta T_{1/2}$, °C	T_p, °C	$\Delta_{app}H$, kJ/mol	Δ_{VH} H, kJ/mol	$\Delta_{app}H$, $\Delta_{VH}H$
3.5%	2.5	10.2	89.4	316	428	0.74
	5	10.6	89.7	307	413	0.74
	7.5	10.75	91.975	316	412	0.77
	10	11.4	92.1	332	389	0.85
	12.5	12	92.4	316	370	0.85
8.8%	2	7.5	86.85	258	574	0.45
	5	9	88.45	269	483	0.56
	7.5	9.4	89.2	293	464	0.63
	10	10.5	90.6	313	417	0.75
	12.5	11.5	92.1	304	383	0.79
	15	11.5	93.2	299	304	0.78
10.8%	2.5	6.9	86.1	247	621	0.40
	5	7.5	86.9	276	577	0.48
	7.5	8.1	87.8	307	534	0.57
	10	9	88.7	324	483	0.67
	12.5	10.55	89	324	456	0.71
24.0%	2	7.2	83.4	233	586	0.40
	5	7.2	84.5	238	590	0.40
	7.5	7.6	85.7	230	563	0.41
	10	7.6	86.4	240	565	0.43
	12.5	7.7	87.2	230	560	0.41
	20	7.7	88.1	238	563	0.42

To check the validity of the kinetic parameters deduced from the theory of Borchardt we have calculated the half-reaction times. Areas under the peaks obtained from samples previously heat-treated at 82.5° during a time equal to the calculated half-reaction time were generally in fair agreement with the experimental ones (Table 3). Nevertheless at 3.5% concentration a straight baseline gives overestimated reaction rates but a sigmoidal baseline gives good results. This may be due to the change in heat capacity playing an important role in that case and not taken account by a straight baseline. This change in the heat capacity is clearly demonstrated in Fig. 4 by the different

slopes of the baseline below and above the denaturation process. The kinetic data obtained are quite different when experimental curves are evaluated with water filled reference pans compared with those obtained from the isothermic method, particulary at 3.5% concentration. The higher value of the half-reaction time (17 min at 82.5°) implies that this heating time favoured aggregation phenomenon even for this relatively low concentration.

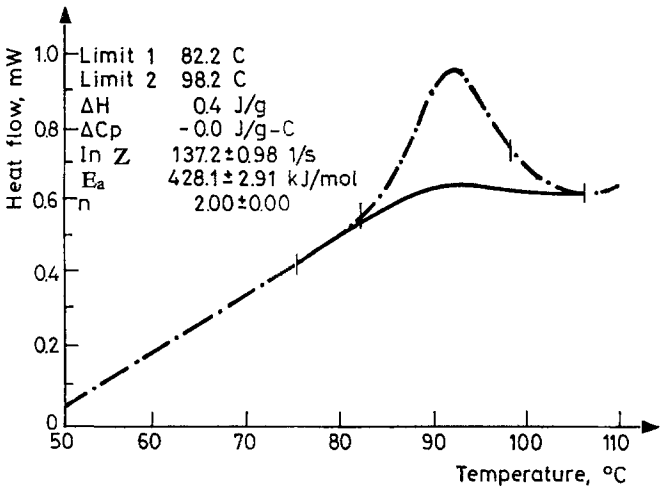

Fig. 4 DSC curve with a sigmoidal baseline. Protein concentration 3.5%, sample mass 60 mg, scan rate 7.5 deg/min

Table 2 Kinetic parameters (a) From Kissinger equation $\ln(1/T_p^2)\,(dT/dt) = -E_A/RT_p + \ln(RZ/E_A)$, (b) From Borchardt method's with a sigmoidal baseline, (c) From Borchardt method's with a straight baseline

Kinetic parameters	E_A, kJ/M	ln Z, s^{-1}	n	$t_{1/2}$ (82.5°C), min	E_A, kJ/M	ln Z, s^{-1}	n	$t_{1/2}$ (82.5°C), min
Method Concentration	(a)	(b)			(c)			
3.5%	409	435 140	2.1	17	392	127	2	6
8.8%	465	458 149	2	6.6	465	152	2	4.9
10.8%	555	553 181.9	1.87	3	497	163	2	2.9
24.0%	557	522 173	1.72	0.5	493	164	1.8	0.4

Figure 5 shows an example of thermograms obtained from β-lg sample (3.5%, 7.5 deg/min) without previous heat-treatment and heat-treated at 85°–6 min and at 82.5°–17 min, respectively. The time intervals correspond

Table 3 Reaction rate (%) after previous heat-treatment of β-lg samples (empty pan as reference)
(a) from isothermic method, $\% = \frac{\Delta_0 H - \Delta H}{\Delta_0 H}$
(b) from Borchardt and Daniels kinetic parameters (see Table 2)

Concentration (scanning rate)	Heat treatment	ΔH J/g	% reaction rate	
			(a)	(b)
3.5% (7.5 deg/min)	no previous heat treatment (ΔH_0)	17.15	0	0
	17 min (82.5°C)	8.6	50	50*, 70.4**
	6 min (85°C)	8.6	50	50*, 68.1**
8.8% (10 deg/min)	no previous heat treatment (ΔH_0)	18	0	0
	5 min (82.5°C)	8.1	46.5	42.9*, 50.6**
10.8% (5 deg/min)	No previous heat treatment (ΔH_0)	15.5	0	0
	3 min (82.5°C)	7.9	49.2	50.9*, 50**
24% (5 deg/min)	no previous heat treatment (ΔH_0)	12.1	0	0
	0.5 min (82.5°C)	5	58.7	60
	1 min (80°C)	6.9	43	46.5

* with sigmoidal baseline
** with straight baseline

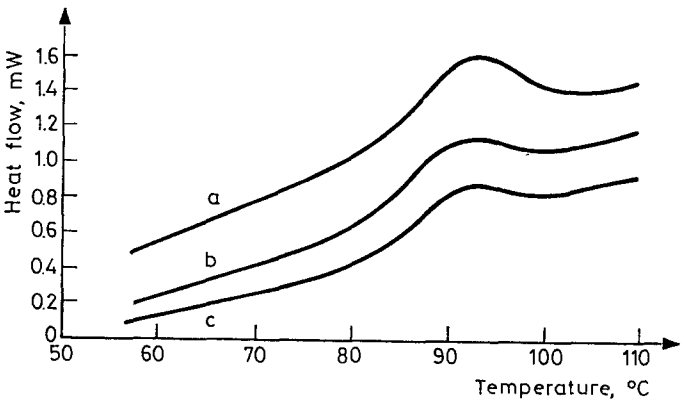

Fig 5 Effect of previously heat-treatment on the peak areas. (3.5%, 7.5 deg/min, empty pan as the reference) 5a. Without previous heat-treatment, 5b. 17 min at 82.5°C

to the calculated half denaturation times at the respective temperatures. Area under peaks of the Figures 5b and 5c are both equal to about half of the thermogram peak from protein sample without previous heat treatment.

Conclusion

The thermal behavior of β-lg (denaturation + aggregation) is concentration dependent at least for concentrations in the range 3.5%−24% and it follows a second order kinetic.

For the lower concentration the denaturation process is at least partially reversible and for the higher concentration the aggregation process dominates and implies the irreversibility of the reaction.

From this complementary study of β-lg denaturation we confirm the hypothetical reaction mechanism proposed in our latter work [1]:

native β-lg----------part. denat. β-lg initiation

step 1: partial denaturation

2 part. denat. β-lg----------(part. denat. β-lg)$_2$

step 2: hydrophobic interactions propagation

(part. denat. β-lg)$_2$----------2 denat. β-lg

and completion of denaturation

n denat. β-lg----------(denat. β-lg)$_n$ termination

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Zusammenfassung – Das thermische Verhalten von β-Lactoglobulin (β-lg), das in destilliertem Wasser bei pH = 3.2 dispergiert ist, wurde für Konzentrationen zwischen 3.5 und 24% im Temperaturbereich von 20–120°C mittels DSC untersucht. In der vorliegenden Arbeit werden thermodynamische Daten des Denaturierungsprozesses ermittelt, wobei sowohl die Methode nach Kissinger als auch diejenige nach Borchardt-Daniels angewendet wurden. Insbesondere wird der Einfluss der Basislinie als auch unterschiedlicher Arten der thermischen Behandlung auf die Resultate behandelt.