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COMPARISON OF THE RESULTS OF THERMAL DENATURATION OF β-LACTOGLOBULIN OBTAINED BY DSC AND UV-SPECTROSCOPY

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Abstract

The thermal denaturation of β-lactoglobulin in the presence of urea and alkylurea solutions were measured. In the presence of a high concentration of urea this protein shows not only heat but also cold denaturation. For studying the effect of temperature two methods were used, differential scanning calorimetry (DSC) and UV-spectroscopy. DSC provides direct model-independent determination of the transition enthalpy in comparison with UV-spectroscopy, which gives only apparent or van't Hoff enthalpy of transition. The UV-melting curves were analyzed on the basis of a two-state approximation. The apparent standard enthalpies of thermal denaturation, $\Delta H^{\circ}_{app.}$, were compared with calorimetric ones.

Keywords: β-lactoglobulin, cold denaturation, DSC, thermal stability, urea, UV-spectroscopy

Introduction

The subject of the study is the thermal denaturation of β-lactoglobulin in the presence of urea and alkylurea solutions. The part of these results were published in a previous paper [1]. It is known that β-lactoglobulin in the presence of high concentration of urea and guanidine hydrochloride (GuHCl) shows not only heat but also cold denaturation [2 – 4]. GuHCl and urea are known to play the following roles: They lower the stability curve so that the low-temperature instability is brought into the range of experimental accessibility, they accelerate the unfolding reaction and they lower the freezing temperature of water so that the subzero temperature range may be explored [5].

Experimental

For studying the effect of temperature two methods, differential scanning calorimetry (DSC) and UV-spectroscopy, were used. Equilibrium thermal unfolding of β-lactoglobulin was monitored calorimetrically with Bio-DSC micro-

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calorimeter of SETERAM (Caluire, France) and spectrophotometrically at 293 nm on a Cary 1 UV–VIS spectrophotometer. The heating rate in experiments was 0.5 deg·min^{-1}. Concentration of protein in water was determined at room temperature by using $E_{1cm}^{1\%w} = 9.6$ at 278 nm.

Results and discussion

DSC curves for β-lactoglobulin in 4*M* aqueous urea solutions at two different *pH* were obtained in the range from 0 to 50°C and vice versa with the purpose of following the pattern of cold denaturation. The results are given in Table 1.

Table 1 Thermodynamic characteristics of β-lactoglobulin cold denaturation (C) and renaturation (R) obtained by DSC in 4*M* aqueous urea solutions at two different *pH*. T_d is the temperature of the transition, ΔH_d is the enthalpy of the transition and ΔC_p is the heat capacity change

	T_d / °C	ΔH_d / kJ·mol^{-1}	ΔC_p / kJ·mol^{-1}K^{-1}
4 M urea (*pH* 7.5)			
C	6.5	-116	8.2
R	13.0	120	-9.6
4 M urea (*pH* 2.5)			
R	12.5	148	-12.1

The UV spectroscopy data were analyzed on the basis of a two-state approximation of protein conformational transition to obtain apparent standard or van't Hoff enthalpy of unfolding, ΔH°_{app}, by using the expression [6],

$$\Delta H^{\circ}_{app} = 4RT^2_{1/2}\left(\frac{\partial f_u}{\partial T}\right)_{T=T_{1/2}}$$

where f_U is the fraction of unfolded protein, R is the universal gas constant and $T_{1/2}$ is the temperature at half-transition where f_U is 0.5.

The results obtained by using UV-spectroscopy for monitoring the thermally induced protein conformation transitions in the presence of different concentration of urea in the temperature range from 3 to 80 °C are given in Fig. 1 and Table 2.

In the Tables 1 and 2 we can see that the cold denaturation (DSC measurements) and cold renaturation are completely reversible reactions only in the

case that protein is not heated over 50°C. It is well known that calorimetric measurements provide a direct model-independent determination of the transition enthalpy. For this reason comparison of the model-dependent van't Hoff transition enthalpy with model-independent calorimetric enthalpy provides insight into the nature of the transition [6, 7]. Recently, it has been shown that the ratio of the van't Hoff and calorimetric enthalpies for heat denaturation of β-lactoglobulin in urea and alkylurea solutions as always smaller than 1 [1]. The disagreement between the two enthalpies does indicate that the heat denaturation of β-lactoglobulin cannot be regarded as a two-state transition, e. i., that the concentration of states intermediate between native and denatured is not negligibly small or that the local conformational changes of tryptophan reflected in UV-melting curves may not accurately monitor the global denaturation event. Cold denaturation shows significantly different behaviour. The agreement between calorimetrically obtained enthalpy and van't Hoff enthalpy of renaturation is much better and this means that the cold denaturation could be regarded in first approximation as a two-state transition [5, 8].

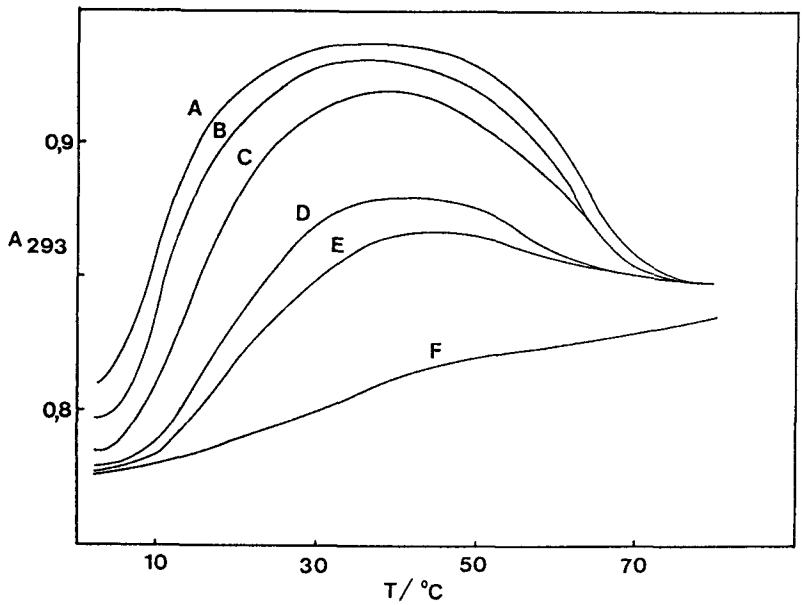

Fig. 1 UV-melting curves of β-lactoglobulin (0.2%) at different urea concentrations in the temperature range from 3 to 80°C measured at 293 nm. A - 3 *M* urea, B - 3.5 *M*, C - 4.0 *M*, D - 4.5 *M*, E - 5.0 *M* and F - 7.0 *M* urea

The change in heat capacity between two states observed at cold denaturation process is much larger than that found for heat denaturation [1]. Similar observation has been reported by other authors [3, 4].

Table 2 Thermodynamic characteristics of β-lactoglobulin cold renaturation obtained by UV–spectroscopy in aqueous urea solutions and reversibility test of the β-lactoglobulin heated up to 40 and 80 °C. 1st column: renaturation data of previously not heated sample; 2nd and 3rd: renaturation data of sample previously heated up to 40 and 80 °C, respectively.

	1		2		3	
Urea conc.(*M*)	$T_{1/2}$ °C	ΔH°_{app} kJmol^{-1}	$T_{1/2}$ °C	ΔH°_{app} kJmol^{-1}	$T_{1/2}$ °C	ΔH°_{app} kJmol^{-1}
3.0	10.9	180				
3.5	12.8	167	13.7	170	18.5	112
4.0	15.9	153	17.2	128	21.2	101
4.5	18.8	122	19.7	118	23.0	80
5.0	21.0	106				

The relative error is estimated to be about 10%.

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Zusammenfassung — Es wird die thermische Denaturierung von β-Laktoglobulin in Gegenwart von Harnstoff- und Alkylharnstofflösungen vermessen. In Gegenwart einer hohen Harnstoff-konzentration denaturiert dieses Eiweiß nicht nur in der Wärme, sondern auch in kaltem Zustand. Zur Untersuchung des Einflusses der Temperatur wurden DSC und UV-Spektroskopie verwendet. Im Vergleich zur UV-Spektroskopie, welche lediglich die scheinbare oder die van't Hoff'sche Umwandlungsenthalpie ergeben, liefert DSC eine direkte modellunabhängige Bestimmung der Umwandlungsenthalpie. Die UV-Schmelzkurven wurden anhand einer Zweizustands-Näherung analysiert. Die scheinbaren Standardenthalpien der thermischen Denaturierung $\Delta H^{\circ}_{app.}$ wurden mit den kalorimetrischen Werten verglichen.