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COMPARATIVE ANALYSIS OF THERMAL PROPERTIES OF TWO TYPES OF β-LACTOGLOBULIN A AND B

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ABSTRACT

In this paper we have adressed to a comparative analysis of thermal denaturation properties of β-lactoglobulin types A and B. The analysis has been carried out in the absence and presence of some osmolytes and polyols with various concentrations at pH= 2.0. Our interpretation showed that the ΔG_D° protein is function of sugar concentration and increased with increasing sugar concentration . On the other hand, ΔH_m of two types lactaglobulin has an insignificant dependence on the sugar concentrations . Estimated denaturation temperatures are 351.0 K and 348.2 K for A type and B type respectively.

KEYWORDS: Protein Stability, Sugar Osmolytes, Thermal Denaturation, β-Lactoglobulin Types A and B

ABBREVIATIONS

β-lgA, β-lactoglobulin A; β-lgB, β-lactoglobulin B; ε, molar absorption coefficient; $\Delta\varepsilon_{293}$, difference molar absorbance at 293 nm ; ΔG_D, Gibbs free energy change of denaturation; ΔG_D^{o}, Standard Gibbs free energy change at 25 °C; ΔC_p, constant-pressure heat capacity change; T_m,midpoint of thermal denaturation; ΔH_m, enthalpy change at T_m; DSC, differential scanning calorimetry.

INTRODUCTION

As we know β-lactoglobulin is deminant whey protein of bovine milk with known primary, secondary and tertiary structures, that its biological function is still fairly unknown [1].

In 1955, it was found that bovine β-lg exists in two genetic forms that differs slightly in their electrophoretic behaviour on paper at pH 8.6, named β-lactoglobulin A (β-lgA) and β-lactoglobulin B (β-lgB) [2], that are predominant types. Variant A differs in amino acid sequence from variant B at position 64 (AspA→GlyB) and 118 (ValA→AlaB). These differences result in distinct biophysical and biochemical properties of the variants, such as heat stability, self association properties and solubility [3].

Among several osmolytes, sugars have been known to stabilize the protein conformation against chemical denaturation or reaction, thermal denaturation, and loss of their biological activity, which can be caused by an increase in temperature, a change in pH value and the addition of various chemicals [4]. Sugars that belong to a class of osmolytes that are synthesized in the bodies to protect organisms against the stresses of high osmotic pressure and freezing. Indeed , sugar synthesis is a good example of a defensive reaction of many organisms. Sugars are commonly employed in freeze-drying formulations of therapeutic proteins to preserve their activity [5].

In the literatures, we find that osmolytes such as sugar and polyols affect the denaturation and together have a

stabilizing effect, increasing thermal denaturation temperature of β-lg and other globular proteins [6-15]. The interaction of β-lgA and β-lgB with some sugar osmolytes is carried out by our team previously [16,17].

In the present article the roles of trehalose, sucrose and sorbitol as sugar osmolytes on the thermal stability of β-lg A [17] and β-lg B [16] are compared. The basic equation were used are:

$$y(T) = \frac{y_N(T) + y_N(T)\exp[-\frac{\Delta H_m}{R}(\frac{1}{T} - \frac{1}{T_m})]}{1 + \exp[-\frac{\Delta H_m}{R}(\frac{1}{T} - \frac{1}{T_m})]} \quad (1)$$

where $y(T)$ is the optical property at temperature $T(K)$, $y_N(T)$ and $y_D(T)$ are the optical properties of the native and denatured protein molecules at T, respectively, R is the gas constant, (T_m) the midpoint of the transition curve and(ΔH_m) is the enthalpy change upon denaturation at T_m [18] .

In the analysis of the transition curve, it was assumed that a parabolic function describes the dependence of the optical properties of the native and denatured protein molecules [19,20].

A plot of ΔH_m versus T_m gives the value of ΔC_p, the temperature-independent heat capacity change at constant pressure. $\Delta G_D(T)$, the value of Gibbs Free Energy change at any temperature T can be estimated using Gibbs–Helmholtz equation with values of T_m, ΔH_m and ΔC_p,

$$\Delta G_D(T) = \Delta H_m(1 - \frac{T}{T_m}) - \Delta C_P[(T_m - T) + T\ln\frac{T}{T_m}] \quad (2)$$

DISCUSSIONS

All thermodynamic quantities from our previous works [16,17] are given in Tables. 1 and 2 were obtained from the analysis of heat denaturation curves of β-lg A and B in the presence and absence of different sugars. In order to see whether the thermodynamically stable intermediate state is observed during the thermal denaturation of β-lg A and B at pH 2.0 and 25 °C , we have followed this denaturation using a probe namely $\Delta\varepsilon_{293}$.

This analysis according to eq. (1) assumes that the transition between the native and denatured states is a two-state process. Most authors state that the unfolding β-lg can be represented by a two states reversible transition between native and unfolded states N→U in the presence of osmolytes, polyols and etc. [21-27].

It can be seen that y_D has a stronger dependency on temperature and osmolyte concentration than y_N, suggesting that osmolytes are more effective on the denatured state of β-lg A and B.

In other words, osmolytes affect the denatured state of the protein more than its native state, leading to a change in protein stability. This case is more obvious for sorbitol and sucrose than for trehalose. It seems that the effect of trehalose follows another mechanism.

Calculated denaturation temperatures show that T_m for β-lg A and B in buffer are 351.0 K and 348.2 K, respectively. This result is in good agreement with the data reported by Divsalar that showed the T_m of native β-lgA are greater than of native β-lgB [33] and the studies of Apenten and Galani [24,28] who gave value 81.2 ◦C for β-lg in 0.05M glycine–HCl buffer pH 2.6**.** The values of ΔG_D^o have been determined by substtution of corresponding values of ΔH_m, Tm and ΔC_p into eq. (3).

$$\Delta G = \Delta H_m (1 - \frac{T}{T_m}) - \Delta C_P [(T_m - T) + T \ln \frac{T}{T_m}] \quad (3)$$

The ΔG_D^o values of native β-lgA are greater than those of native β-lgB. Thus, it can be concluded that native β-lgA has a higher thermal stability relative to native β-lgB. These data are in a good agreement with previous reports which suggest that the difference in the thermal behavior of β-lgA and β-lgB can be explained by the destabilization of the core of the β-lgB relative to β-lg -A, leaving a cavity formed by the loss of the two methyl groups as a result of the substitution ValA→AlaB [1, 24]. Greater stability is predicted for β-lgA by this analysis because β-lgA has more hydrophobic residues than β-lgB (due to the substitution Val/Ala in β-lgA / β-lgB).

It can be seen from tables 1 and 2 [16,17] that T_m of β-lgA and B at pH 2.0 increase linearly with an increase in the concentration of individual sugar. But the ΔH_m values of many proteins remain unchanged in the presence of various osmolytes [29-35]. We have also observed that the ΔH_m of β-lg A and B in the presence of different sugars shows insignificant dependence on type and concentration of the sugar. This and earlier observations suggest that sugar osmolytes have no significant affinity on the protein.

We have determined ΔC_P (= $(\delta \Delta H_m / \delta T_m)p$) from the linear plot of ΔH_m and T_m values at pH 2.0. Values of ΔCp in the presence of different concentrations of sugars are 5.39 and 5.3 for β-lg A and B, respectively[16,17].

A DSC (differential scanning calorimetry) study of thermal and cold denaturation of β-lg was reported that in aqueous solutions at pH 2.0 (0.1 *M* KCl/HCl) ΔC_P=5.58 ± 0.7 kJ $mol^{-1}K^{-1}$ [36]. Since β-lg A has only two more CH_2, than β-lg B, hence increasing in ΔC_P, is reasonable. Model compound data indicate that each CH_2 group which is transferred from a nonpolar environment to water should contribute about 16±3 cal. $mol^{-1}K^{-1}$ to ΔC_P [37]. These results are in good agreement with Steve A.S et al report [38]. It is seen that there are a very small decrease in ΔC_P of β-lg A and B in various sugars. This is in a good agreement with those reported earlier [31, 8 and 39]. The effect of sugars on protein stability have been explained by other groups [40-43]. Both Timasheff's and Bolen's groups have argued that the source of stabilization of protein by sugars is due to the shifting of denaturation equilibrium towards the N state [35, 39]. Thus, the effects of co-solvents on the denaturation equilibrium, N state ↔ D state under the native condition will be known only by measuring ΔG_D^o. It seems from Tables 1 and 2 that the effect of sugars on ΔG_D^o of protein increases with increasing sugar concentrations at pH 2.0. It is seen that the $\%\Delta\Delta G_D^o$ increases with the molar concentration of the additive.

The earliest thermodynamic mechanism of stabilization [44] suggests that the saccharides, sucrose, and trehalose, exert their effects differently than do the other protecting osmolytes. These osmolytes favourably interact with the peptide unit. Concomitantly, the peptide unit becomes excessively hydrated. The net effect of trehalose is a large hydration of the peptide unit with a net-zero salvation by trehalose. Sucrose is even more enriched around peptide groups. Along with the additional positive hydration, this enrichment causes the local density of the solution to strongly increase around the peptide unit. In fact, the positive solvation of the peptide unit by sucrose is comparable with the enrichment of urea around the peptide unit. Sucrose, however, brings additional waters of hydration to the peptide unit, whereas urea excludes waters of hydration. Overall, these results give a much insight into the solvation effects that govern the stability of proteins in osmolyte solutions.

Poddar and co-workers [45] estimated enthalpy and entropy contributions to ΔG_D^o in a given solvent condition using the values of ΔT_m, ΔH_m (given in Tables 1 and 2) and ΔC_p in equations,

$$\Delta H_D^o = \Delta H_m - \Delta C_p (T_m - 298.15) \quad (4)$$

and

$$\Delta S_D^o = (\Delta H_m/T_m) + \Delta C_p \ln (298.15/T_m) \quad (5)$$

The resultant values of equations 3 and 4 are given in Table 3. It seems that both ΔH_D^o and ΔS_D^o are positive and $\Delta H_D^o > T\Delta S_D^o$. Thus the stabilization of β-lactoglobulins A and B by sugars are under enthalpic control.

Lee and Timasheff [39] have reported that the stabilization of α-chymotrypsin and chymotrypsinogen by sucrose is also under enthalpic control. Podar and co-workers [45] also showed stabilization of RNase-A by sugars is under enthalpic control.

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APPENDICES

Table 1: Stability Parameters of β-lgA in the Presence of Various Concentrations of Sugar Osmolytes and Polyols at pH 2.0[17]

Osmolytes	M	T_m/K	Δ T_m/K	ΔH_m(kJ.mol^{-1})	$\Delta\Delta H_m$(kJ.mol^{-1})	ΔG_D^o(kJ.mol^{-1})	$\%\Delta\Delta G_D^o$
Control	0.00	351.0	0.0	448.2	0.00	44.9	0.00
Trehalose	0.25	351.9	0.9	450.1	1.90	45.4	1.10
	0.50	353.0	2.0	453.1	4.90	46.1	2.70
Sucrose	0.25	351.8	0.8	450.0	1.80	45.4	1.10
	0.50	353.3	2.3	455.0	6.80	46.5	3.60
	0.75	355.1	4.1	464.5	16.3	48.4	7.80
	1.0	356.5	5.5	479.2	31.0	51.2	14.0
Sorbitol	0.25	351.7	0.7	450.0	1.80	45.3	0.90
	0.5	353.1	2.1	453.7	5.50	46.3	3.10
	0.75	354.8	3.8	462.2	14.0	48.0	6.90
	1.0	356.0	5.0	469.4	21.2	49.2	9.60

Table 2: Stability Parameters of β-lgB in the Presence of Various Concentrations of Sugar Osmolytes and Polyols at pH 2.0 [16]

Osmolytes	M	T_m/K	Δ T_m/K	ΔH_m(kJ.mol^{-1})	$\Delta\Delta H_m$(kJ.mol^{-1})	ΔG_D^o(kJ.mol^{-1})	$\%\Delta\Delta G_D^o$
Control	0.00	348.2	0.0	411.2	0.00	39.9	0.00
Trehalose	0.25	350.2	2.0	416.9	5.70	40.4	1.30
	0.50	352	3.8	424.7	14.0	41.9	5.00
Sucrose	0.25	349.7	1.5	414.8	3.60	40.3	1.00
	0.50	351.5	3.3	422.4	11.0	41.5	4.00
	0.75	352.6	4.4	427.7	17.0	42.5	6.50
	1.0	354.6	6.4	443.1	32.0	45.3	14.0
Sorbitol	0.25	349.6	1.4	414.2	3.00	40.1	0.50
	0.50	351.4	3.2	421.9	11.0	41.4	3.80
	0.75	352.7	4.5	428.0	17.0	42.4	6.30
	1.0	354	5.8	435.5	24.0	43.9	10.0

Table 3: Stability Parameters of β-Lactoglobulins A and B in the Presence of 1M Sugar at pH 2.0 and 25 ◦C (all of the Reported Quantities are in the United of kJ.mol^{-1})

Sugars 1M	β-Lactoglobulin A			β-Lactoglobulin B		
	ΔH_D^o	$T\Delta S_D^o$	ΔG_D^o	ΔH_D^o	$T\Delta S_D^o$	ΔG_D^o
Control	168.1	123.2	44.9	145.8	105.9	39.9
Sucrose	169.9	118.3	51.6	143.9	98.5	45.4
Sorbitol	162.8	112.9	49.9	139.5	95.4	44.1