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COMPARATIVE DSC STUDY OF HUMAN AND BOVINE SERUM ALBUMIN

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The thermal denaturation process of bovine and human both fatty acid containing and fatty acid free albumins in aqueous solution was studied by use of differential scanning calorimetry. Human serum albumins were found to be more stable than their bovine counterparts. Fatty acid free albumins were characterized as generally less stable, more susceptible to aggregation, their unfolding endothermic transition was less cooperative and with the smaller degree of reversibility. Deconvolution analysis with using a non-two-state model with two component transitions showed essential differences in the thermodynamic parameters between all studied albumins, particularly regarding the high-temperature component transition.

Keywords: *bovine serum albumin, DSC, human serum albumin, thermal stability*

Introduction

Albumins from different mammalian species show many similarities in the physico-chemical properties [1, 2]. There are 332 (57%) invariant residues in the sequences of mammalian albumins. 76% of sequence identities have been noted between bovine (BSA) and human (HSA) albumins. These two albumins are indistinguishable by some physical criteria and similar taking into account others e.g. surface hydrophobicity properties, partitioning behaviour in some aqueous two-phase system [3]. On the other hand serious differences in electrophoretic behaviour [4], thermal and chemical stability [5], binding and photochemical properties [6, 7] exist between different mammalian albumins.

Application of albumin in various medical areas is widely known [8]. Past studies have shown some new uses of albumin in clinical practice, for instance as a fatty acid carrier for biosynthesis of lens lipids [9], solders for laser tissue welding [10–12]. Different species of albumin have been used interchangeably when performing laser welding research. Bleustein *et al.* [10, 11] show that species-specific and fatty acid-specific differences exist when albumin solders are used to increase wound strength and increase the consistency of repairs. Currently, two species of albumin, bovine and human, are being primarily used for this purpose.

Recent improvements in sensitivity and usability of DSC instrumentation, enable the detailed investigation of the thermal properties of protein. The interspecies as well as the environmental differences between various forms of protein can be easily detected.

DSC thermal transitions of BSA and BSAf were studied by us in detail earlier [13]. In this study the similar investigations are carried out for human albumin and the comparison of the thermal unfolding properties of bovine (BSA) and human (HSA) both fatty acid containing and fatty acid free albumins are made.

Materials and methods

Two species of albumin, bovine and human both fatty acid containing (BSA, lot 79H7614 and HSA, lot 111K7612) and fatty acid free (BSAf, lot 89H7604, HSAf, lot 113K7601), essentially globulin free (purity minimum 99%) were purchased from Sigma. DSC measurements were carried out in the temperature range 20–100°C by using the ultrasensitive microcalorimeter (VP DSC, MicroCal Inc., Northampton, MA). A scan rate 60°C h^{-1} was chosen for the present study, except for the experiment on scan-rate dependence, where the scan rates ranged from 40 to 90°C h^{-1}. Albumin solutions with concentrations ranging from 1 to 10 mg mL^{-1} were prepared by direct dilution of the lyophilised protein with sterile water. pH of the albumin solutions was 6.0±0.5. All other DSC experimental details were the same as described previously [13].

DSC curves were analysed with MicroCal Origin software. The calorimetric data were corrected for the calorimetric baseline (by subtracting water – water scan) and for the difference in heat capacity between the initial and the final state by using a sigmoidal baseline.

Statistical analysis of the results was done with Statistica 5.1 using Kruskal-Wallis test.

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Results and discussion

The DSC profiles of the defatted and undefatted HSA and BSA solutions (concentration 3 mg mL^{-1}) recorded at a scan rate of 40°C h^{-1} are shown in Fig. 1. All observed endothermic transitions are broad, with a positive value for the change in heat capacity ΔC_p. However it is well seen that the shape of the peaks connected with albumin denaturation process differs essentially. The transition for BSAf is very broad and bimodal while that for BSA single and much more narrow. Peaks with shoulders at the left and right side are visible for HSA and HSAf respectively in Fig. 1. The transition temperatures T_m (defined as the temperature at which a local maximum occurs in the excess heat capacity, C_{ex}) are lower for both fatty acids free albumins than for their nondefatted counterparts.

The concentration and scan rate effects on human albumin DSC curves are shown in Figs 2 and 3 respectively. The shape of the transitions appears to depend on the protein concentration as well as on the scan rate. HSA transition becomes bimodal at high concentration or low scan rate (Figs 2A, 3A). A single endotherm with a shoulder at the right side of the peak is observed for HSAf at the highest studied protein concentration. At lower concentration the DSC transition seems to consist of two or even three component peaks (Fig. 2B). HSAf curves are dependent on scan rate. At the lowest scan rate (40°C h^{-1}) the endothermic peak is followed by an exothermic one at higher temperatures (Fig. 3B). This exothermic peak is probably connected with albumin aggregation.

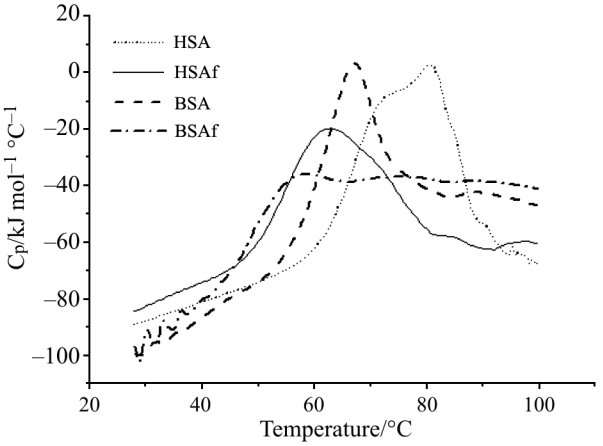

Fig. 1 DSC profiles of the defatted and undefatted HSA and BSA solutions (concentration 3 mg mL^{-1}) recorded at a scan rate of 40°C h^{-1}

The results of preliminary analysis of albumin DSC curves are listed in Tables 1 and 2 (transition temperatures – T_m, enthalpy changes – ΔH) and shown in Fig. 4 (the widths of curves at half height – HHW). Reported values are the means of 3–4 independent replicates. The average standard deviations of T_m and ΔH are 0.4°C, 48 kJ mol^{-1} in Table 1 and 0.8°C, 80 kJ mol^{-1} in Table 2 respectively. The average T_m for HSA equal 81.3 (with SE=0.2) is about 11°C higher than for BSA and about 20°C higher than for HSAf. The average T_m=61.4°C (with SE=0.4) for HSAf is somewhat smaller than the value 63.1±0.4°C at pH 7.4 reported by Pico [14] and 65.7°C found by Ross and Shrake [15] for similar concentration range at pH 7.7 in 150 mM NaCl. T_m for BSAf is about 5°C lower than for HSAf and about 14°C lower than for BSA. For undefatted albumins T_m increase with increasing protein concentration and practically does not depend on scan rate. For fatty acid free albumins T_m slightly increases with scan rate increasing, however the differences are not statistically essential. The denaturation enthalpy ΔH does not depend on protein concentration and scan rate for human albumins and increases slightly with increasing protein concentration for bovine albumins. The average ΔH values are 1127 kJ mol^{-1} (with SE=27) and 871 kJ mol^{-1} (with SE=18) for HSA and HSAf respectively. As illustrates Fig. 4, HHW values decrease rapidly with in-

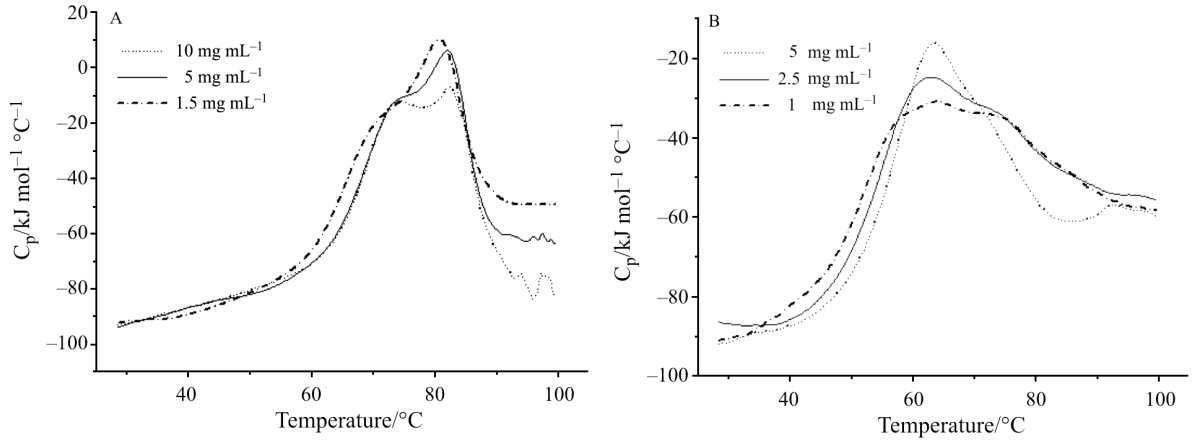

Fig. 2 The concentration effect on human albumin DSC curves (A – HSA, B – HSAf); (scan rate 60°C h^{-1})

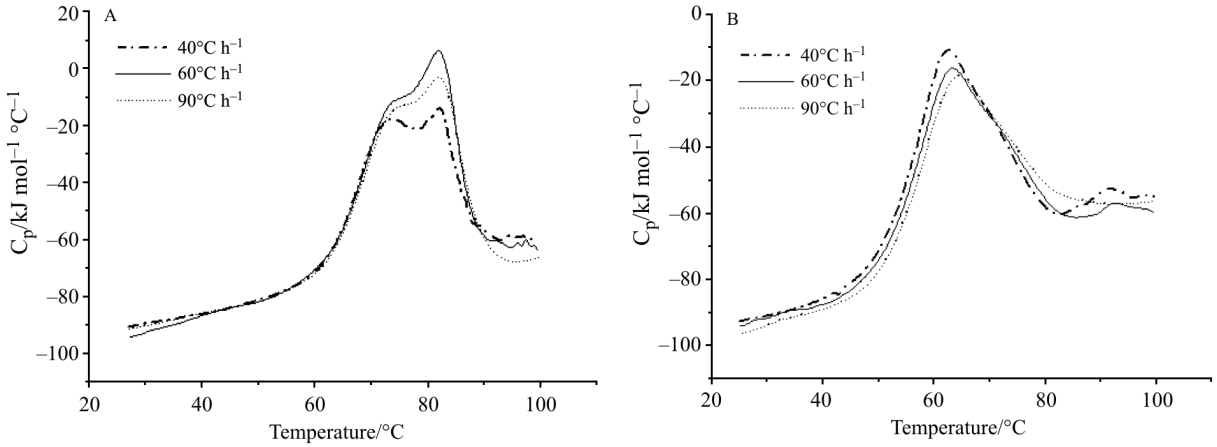

Fig. 3 The scan rate effect on human albumin DSC curves (A – HSA, B – HSAf); (concentration 5 mg mL^{-1})

Table 1 The transition parameters for BSA and HSA from DSC curves at different concentrations and scan rates

Concentration/mg mL^{-1}	Scan rate/°C h^{-1}	T_m/°C		ΔH/kJ mol^{-1}	
		BSA	HSA	BSA	HSA
	90	68.0		947	
2	60		80.5		1199
	40	66.9	80.1	708	1127
	90	69.0	81.3	985	1077
3	60	68.9	80.9	1039	1140
	40	68.8	80.8	1014	1056
	90	70.9	81.8	1098	1165
5	60		81.5		1031
	40	70.5	81.6	1071	934
	90		82.7		1219
10	60		82.4		1152
	40		82.9		1244

Table 2 The transition parameters for BSAf and HSAf from DSC curves at different concentrations and scan rates

Concentration/mg mL^{-1}	Scan rate/°C h^{-1}	T_m/°C		ΔH/kJ mol^{-1}	
		BSAf	HSAf	BSAf	HSAf
	90	57.3	61.3	440	855
2	60		60.5		999
	40	56.8	60.2	453	771
3	60	56.9	63.5	496	817
	90	56.2	63.7	550	880
5	60	56.2	62.5	553	796
	40	55.9	62.0	541	825

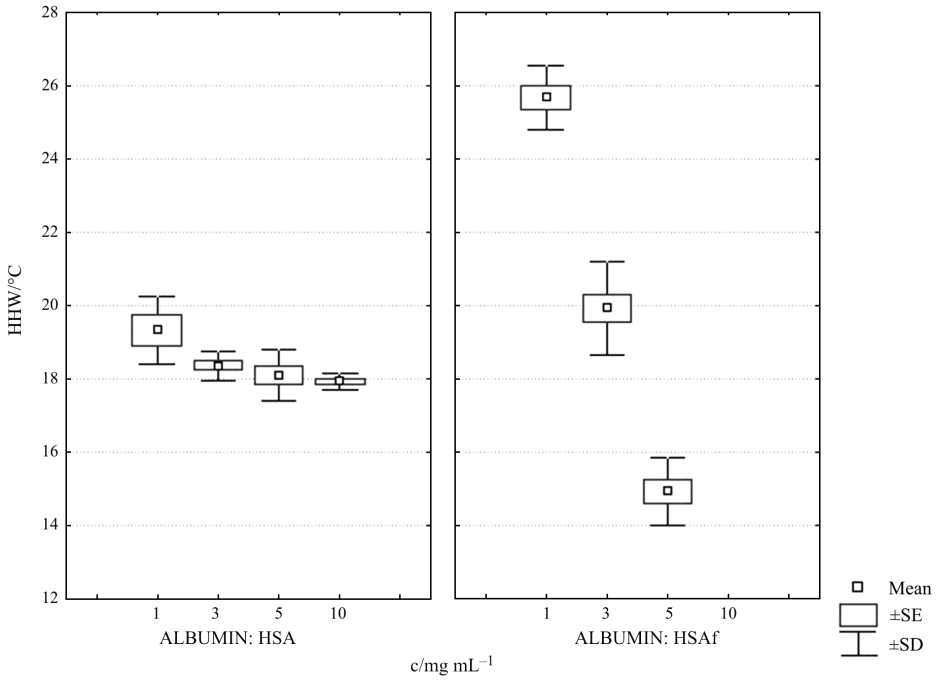

Fig. 4 The widths of curves at half height (HHW) *vs.* albumin concentration

creasing HSAf concentration, thus HSAf thermal denaturation transition is more cooperative at higher albumin concentration. In the case of HSA the values of HHW decrease only slightly with increasing protein concentration.

The reversibility of human albumin denaturation process was evaluated similarly as previously for bovine albumin [13]. In Table 3 the percentage reversibility (±3%) of four kinds of albumin is compared. The denaturation transition is more reversible for human than for bovine albumins and more reversible for nondefatted than for fatty acid free albumins.

Comparison of the thermodynamic parameters obtained for various kinds of albumin from experiments carried out at the same heating rate and protein concentration provides a v˙id indication of relative thermal stability. We found a decrease in the transition temperature T_m and reduction in the enthalpy of denaturation (ΔH) for defatted species, what is consistent with the previous studies [11, 13, 15]. Generally human serum albumins are characterized by higher thermal stability than their bovine counterparts.

The high reversibility of the albumin after heat up to the temperature above T_m (Table 3), only slight

Table 3 Reversibility of bovine and human serum albumin denaturation process after preliminary heating to different temperatures T/°C

T/°C	Reversibility/%			
	BSA	HSA	BSAf	HSAf
60	100	100	90	100
70	85	95	67	90
80	33	70	32	42
100	8	13	7	7

Table 4 The thermodynamic parameters (±SEM) of the two-component transition for the thermal unfolding process of human and bovine albumins in aqueous solutions (concentration 5 mg mL^{-1}), obtained from the fitting process in Non-2-State model

Albumin	T_1/°C	$\Delta H_{cal,1}$/ kJ mol^{-1}	$\Delta H_{vH,1}$/ kJ mol^{-1}	$\Delta H_{cal,1}/\Delta H_{vH,1}$	T_2/°C	$\Delta H_{cal,2}$ kJ mol^{-1}	$\Delta H_{vH,2}$ kJ mol^{-1}	$\Delta H_{cal,2}/\Delta H_{vH,2}$
HSA	73.4±0.2	761±20	265±5	2.9	82.2±0.2	263±31	613±26	0.4
HSAf	62.5±0.3	760±14	251±5	3.0	71.9±0.6	83±15	511±53	0.2
BSA	61.8±0.5	496±24	321±10	1.5	67.3±0.3	503±15	419±25	1.2
BSAf	55.8±0.5	346±18	277±16	1.3	69.6±0.6	176±18	236±20	0.8

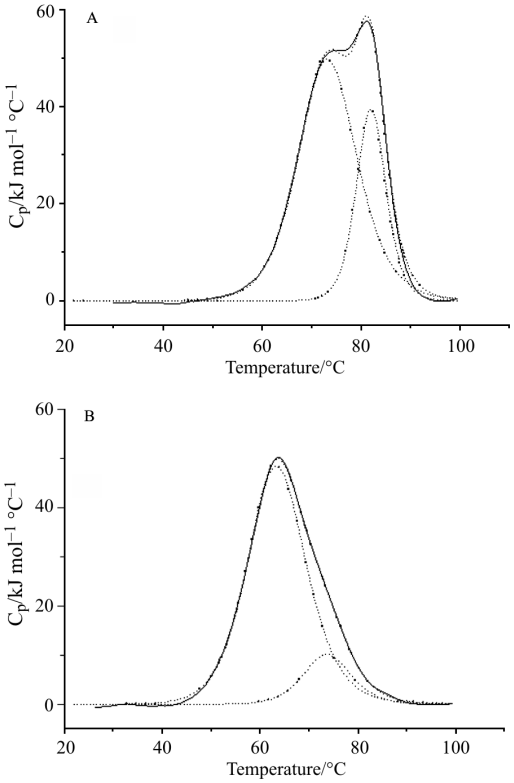

Fig. 5 The curve fitting of DSC profiles for aqueous albumin solution in Non-2-State model: A – HSA, B – HSAf; —– experimental curve, ···· – the result of deconvolution analysis

scan-rate effect on T_m values and the dependence of the DSC curves on protein concentration indicate that albumin thermal unfolding may be considered as equilibrium/dissociation process. It should be noted however that particularly in the case of defatted albumins, which are more susceptible to aggregation, some kinetic distortion occurs.

To obtain detailed information about the thermal unfolding of different kind of albumin molecules a deconvolution of DSC traces were performed with using a non-two-state model with two-component transitions. The results of fitting for HSA and HSAf are shown in Fig. 5 A, B. For BSA and BSAf the fittings of DSC curves with the assumption of such a model were reported earlier [13, 16]. In Table 4 the results for human and bovine albumins are listed. The differences in the thermal unfolding process of the studied forms of albumin are well visible. The comparison of fits for HSA and HSAf in Fig. 5 A, B suggests the apparent differences mainly between the high-temperature component transitions.

Conclusions

Human serum albumins show higher thermal stability than their bovine counterparts. Among studied albumins nondefatted bovine albumin seems to possess the most compact structure in aqueous solution. Not only are there differences between each species' albumin, but differences also exist depending on the presence of fatty acids bound to albumin. Nondefatted albumins unfold in higher temperatures with higher enthalpy change. The lack of fatty acids causes decreasing of the reversibility and cooperativity of the thermal denaturation process. Fatty acid free albumins are also more susceptible to aggregation.

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