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CALORIMETRIC AND VOLUMETRIC DATA OF NUCLEATING BOVINE ALBUMIN SOLUTIONS AT VARIOUS NaCl, Li_2SO_4 AND $(NH_4)_2SO_4$ CONCENTRATIONS

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Heat effects and densities of bovine albumin solutions in Na-acetate buffer pH 4.2 at various NaCl, Li_2SO_4 and $(NH_4)_2SO_4$ concentrations were determined by a LKB 10700-2 microcalorimeter and an Anton Paar 60/602 densimeter (25°C). The density measurements were made after 1 and 48 h of the dissolution of bovine albumin in the buffer. The correlations between the changes of the enthalpy of salting and apparent molar volumes *vs.* concentrations of salts were determined.

Keywords: *bovine albumin, calorimetry, density, salts: Li_2SO_4, NaCl, $(NH_4)_2SO_4$*

Introduction

Predicting the solution conditions where proteins aggregate and successfully crystallize remains a significant obstacle in the advancement of structural molecular biology. Salts are often used in study of precipitation and crystallization of proteins. The mechanism of salting processes have been the subject of investigations by several methods, chemical factors and experimental techniques [1] e.g. quasi-elastic light scattering (QELS), interferometry were used to understand the fundamentals of protein growth, whereas unsaturated dilute protein solutions were often investigated by spectroscopic methods such as UV, fluorescence, CD-spectroscopy, light and small angle X-ray scattering or osmometry were used to find out whether salts have influence on the physical behavior or structure of protein studied. Calorimetric methods were rarely used in these investigations. According to our knowledge only a few papers were published [2–4] before starting by us with investigations devoted to the calorimetric study of the kinetics of lysozyme precipitation [5], determination of thermodynamic parameters (ΔH, K) in salting processes [6] and correlations between enthalpic and volumetric properties of lysozyme solutions of various salts concentrations [7, 8].

In this contribution the results of enthalpic and volumetric investigations of nucleating bovine albumin solutions at various NaCl, Li_2SO_4 and $(NH_4)_2SO_4$ concentrations are presented.

Materials and methods

Bovine albumin (molar mass 66431) was purchased from Sigma (Lot 100K 7415). Suprapure sodium chloride was obtained from Merck (catalogue number 106406). Lithium sulfate and ammonium sulfate were purchased from Fluka Co. (catalogue number 62613 and 09978, respectively). The solutions were prepared by mass in the buffer containing 0.1 M sodium acetate (pH 4.2) at 25°C using distilled and deionized water.

The calorimetric experiments were realized with the use of a batch LKB 10700-2 microcalorimeter. Equal volumes (2 cm^3) of the bovine albumin in the buffer were mixed with electrolyte–buffer solutions. The experiments were carried with different concentrations of electrolytes and constant concentration of bovine albumin. The microcalorimeter was tested by the Joule's effect generated after each calorimetric measurement. It can be mentioned that the measurement of mixing of buffered salt solution into the buffered protein solution (or vice versa) is not enough to determine the enthalpy of salting. This heat effect is the result of superposition of all individual changes occurring in the system. Among other things, they include the effects associated with dilution of interacting components into the buffer solution in which they are dissolved. Thus to determine the net heat of salting it is necessary to introduce corrections. This requires individual measurements to determine the heat effect of dilution when: 1) salt is injected into the buffer, 2) protein is injected into buffer, 3) buffer is injected into buffer. This last heat effect is small and was neglected. The heat of salting Q was calculated as

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equal to $Q=Q_1-Q_2-Q_3$ where Q_1 is total heat effect measured in the calorimeter, Q_2 is heat of dilution of electrolyte in the buffer and Q_3 is heat of dilution of bovine albumin in the buffer. The heat of dilution Q_2 of natrium chloride solution (NaCl) in the buffer were determined experimentally, the heat of dilution Q_2 of Li_2SO_4 and $(NH_4)_2SO_4$ were determined previously [8] and calculated from following relationships:

$$Q_2\ [Li_2SO_4]=0.227+0.727m-0.209m^2\ \text{kJ mol}^{-1}$$
$$Q_2\ [(NH_4)_2SO_4]=-0.013-1.158m+0.529m^2\ \text{kJ mol}^{-1}$$

where m is final concentration of electrolyte. Heat effect of dilution of bovine albumin in the buffer Q_3 was determined experimentally.

The densities of solutions investigated were determined with Anton Paar 60/602 digital densimeter after 1 and 48 h after the dissolution of the bovine albumin in the buffer. The working procedure have been described elsewhere [7]. The uncertainty in density measurements $\delta(d)$, was about $\pm 1\cdot 10^{-5}$ g cm^{-3}, whereas the uncertainty in molality determinations, $\delta(m)$ was $\pm 1\cdot 10^{-5}$ m. The calibration constant of the densimeter were determined from measurements with dry air and deionized water.

Experimental

The obtained calorimetric data Q_1, Q_2, Q_3 and the values of final concentrations of bovine albumin m^f_{alb} and electrolyte m^f_{elect} solutions in the buffer as well as masses of bovine albumin were collected in Table 1. As can be seen from the collected data, the heat of dilution of NaCl and $(NH_4)_2SO_4$ are negative whereas

Table 1 Results of microcalorimetric investigations

m^f_{alb}/mol kg^{-1}	mass$_{alb}$/mol 10^{-6}	m^f_{elect}/mol kg^{-1}	Q_1/J	Q_2/J	Q_3/J
		NaCl			
0.001217	4.49	0.40567	0.162	–0.388	0.074
0.001222	4.51	0.50687	0.008	–0.587	0.074
0.001216	4.42	0.65049	–0.271	–0.924	0.074
0.001233	4.49	0.80915	–0.696	–1.401	0.074
0.001225	4.46	0.92885	–0.963	–1.827	0.074
0.001230	4.41	1.06613	–1.301	–2.228	0.074
0.001241	4.48	1.18644	–1.691	–2.681	0.074
0.001230	4.49	1.27398	–2.017	–3.020	0.074
0.001248	4.43	1.37523	–2.219	–3.210	0.074
0.001254	4.52	1.44876	–2.566	–3.514	0.074
		Li_2SO_4			
0.001219	4.56	0.19412	0.556	0.261	0.074
0.001212	4.46	0.25285	0.647	0.370	0.074
0.001206	4.42	0.34688	0.766	0.577	0.074
0.001214	4.43	0.47718	0.974	0.921	0.074
0.001236	4.49	0.65203	1.497	1.449	0.074
0.001248	4.44	0.93597	2.318	2.421	0.074
0.001251	4.50	1.07720	3.184	2.977	0.074
0.001283	4.61	1.21795	3.888	3.538	0.074
		$(NH_4)_2SO_4$			
0.001234	4.54	0.34485	–0.130	–0.412	0.074
0.001255	4.57	0.46678	–0.451	–0.745	0.074
0.001263	4.56	0.59523	–0.772	–1.109	0.074
0.001258	4.47	0.77033	–1.257	–1.616	0.074
0.001306	4.51	0.92921	–1.676	–2.048	0.074
0.001302	4.53	0.95733	–1.743	–2.121	0.074
0.001313	4.51	1.12236	–2.131	–2.493	0.074
0.001326	4.51	1.33632	–2.644	–2.797	0.074

Table 2 Density of bovine albumin at various electrolyte concentration after 1 and 48 h (25º)

Time/h	m/mol kg^{-1}	d_3/g cm^{-3}	d_2/g cm^{-3}
1	0	1.01579	0.99893
48	0	1.01629	0.99893
		NaCl	
1	0.40803	1.03300	1.01519
48		1.03279	1.01558
1	0.60967	1.03936	1.02294
48		1.03955	1.02308
1	0.81352	1.04631	1.03031
48		1.04696	1.03059
1	1.00356	1.05239	1.03699
48		1.05365	1.03760
1	1.10660	1.05724	1.04115
48		1.05788	1.04133
1	1.21442	1.06075	1.04525
48		1.06088	1.04538
1	1.30243	1.06421	1.04854
48		1.06419	1.04862
1	1.45431	1.06895	1.05423
48		1.06947	1.05418
		Li_2SO_4	
1	0.20577	1.03415	1.01792
48		1.03429	1.01784
1	0.40357	1.05049	1.03490
48	–	1.05029	1.03453
1	0.60348	1.06688	1.05230
48		1.06755	1.05260
1	0.80798	1.08202	1.06927
48		1.08208	1.06944
1	1.10031	1.10281	1.09203
48			1.09229
1	1.20553	1.11119	1.10040
1	1.34967	1.12312	1.11003
48		1.12033	1.10959
		$(NH_4)_2SO_4$	
1	0.60071	1.05686	1.04124
48		1.05718	1.04215
1	0.80283	1.06899	1.05418
48		1.06935	1.05480
1	0.90492	1.07463	1.06059
48		1.07489	1.06047
1	1.00159	1.08022	1.06614
48		1.08031	1.06612
1	1.10330	1.08605	1.07196
48		1.08666	1.07245
1	1.10330	1.08605	1.07196
48		1.08666	1.07245
1	1.15213	1.08846	1.07489
48		1.08874	1.07492
1	1.20027	1.09077	1.07761
48		1.09144	1.07774
1	1.39943	1.10118	1.08869
48		1.10261	1.08938

of Li_2SO_4 is positive. The contribution of the heat of dilution Q_2 values in the total heat effects Q_1 is meaningful. The heat effects Q_3 are constant and characterized by small values in comparison to the Q_1 values.

The results of density determination and calculated from these values apparent molar volumes data are collected in the Table 2, where m is molality of electrolyte, d_3 is density of bovine albumin–buffer–electrolyte solution, d_2 is density of buffer–electrolyte solution and $V_{\phi,3}$ is apparent molar volume of the bovine albumin buffered solutions.

Results and conclusion

As follows from the data presented in Table 1 the calorimetric measurements were done in relatively high spectrum of salts concentrations from around 0.2 to 1.4 mol kg^{-1}. Such a range of concentrations was chosen to determine the enthalpy of salting and apparent molar values characteristic for two opposite phenomena in salting processes [9] namely salting-in at low salt concentrations and salting-out at higher salt concentrations. At low salts concentration ions stabilize proteins in solution via reduction of electrostatic repulsion between charged protein macromolecules, whereas at higher concentration preferential hydration of salt ions can desolvate proteins causing them to aggregate, precipitate or crystallize. These phenomena should be accompanied by significant changes in both enthalpy of salting and apparent molar volumes values. In the calorimetric studies of lysozyme–NaCl system [6] in the course of $\Delta H=f(m\text{NaCl})$ function one could distinguish the region with the minimum enthalpy of salting value, which could be attributed to specific salting-out of the protein. The salt concentration at ΔH_{min} can be treated as characteristic of the ability of salt to precipitate protein. The data of the enthalpy of salting *vs.* concentrations given on Figs 1–3 clearly show the regions in which drastic enthalpy changes occur. They are different for different electrolytes. In the case bovine albumin NaCl solution the values of the enthalpy of salting were about 3 times

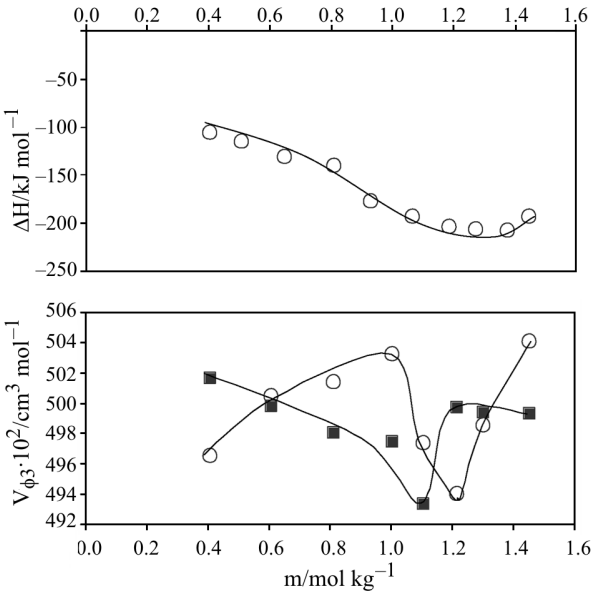

Fig. 1 Comparison of the enthalpy of salting and apparent molar volume data of nucleating bovine albumin solutions at various NaCl concentrations. The data of apparent molar volumes are given at – ○ – 1 h and – ■ – 48 h after the dissolution of the bovine albumin in the buffer with salt

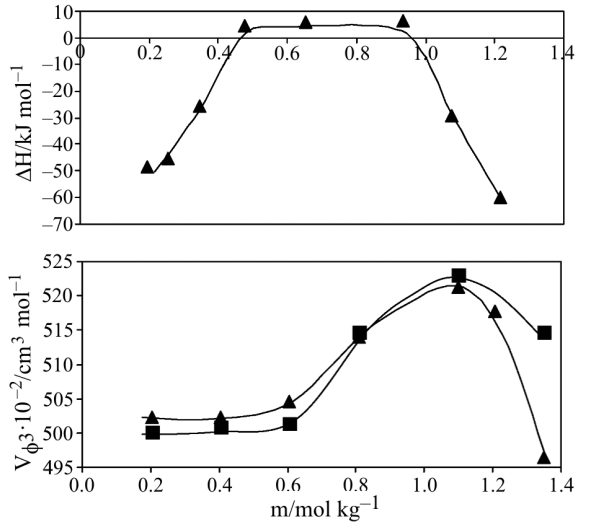

Fig. 2 Comparison of the enthalpy of salting and apparent molar volume data of nucleating bovine albumin solutions at various Li_2SO_4 concentrations. The data of apparent molar volumes are given – ▲ –1 h, and – ■ – 48 h after the dissolution of the bovine albumin in the buffer with salt

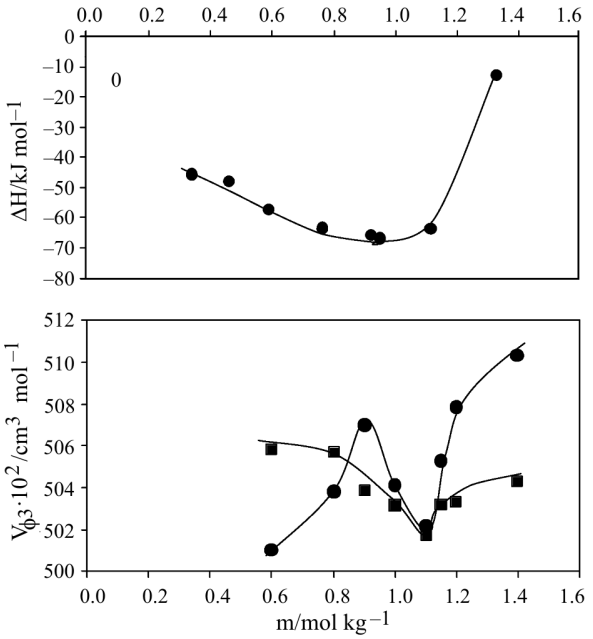

Fig. 3 Comparison of the enthalpy of salting and apparent molar volume data of nucleating bovine albumin solutions at various $(NH_4)_2SO_4$ concentrations. The data of apparent molar volumes are given – ● –1 h and – ■ – 48 h after the dissolution of the bovine albumin in the buffer with salt

higher than those obtained of the some concentration bovine albumin–Li_2SO_4 and bovine albumin–$(NH_4)_2SO_4$ solutions (Table 1).

The values of enthalpy of salting of bovine albumin–NaCl solution and bovine albumin–$(NH_4)_2SO_4$ solution (Figs 1–3) decrease with the increasing salt concentration and reach minimum at 1.37*m* NaCl and 0.95m $(NH_4)_2SO_4$. The enthalpy of salting values for the bovine albumin–Li_2SO_4 solution do not show a minimum in the investigated concentration range; they increase to the 0.95m Li_2SO_4, and then decrease (Fig. 2). For the concentration range studied the course of the enthalpy of salting changes was compared with the changes of apparent molar volumes. At the same NaCl and $(NH_4)_2SO_4$ concentrations, similarly to the enthalpy of salting, the minima of apparent molar volumes occur. For Li_2SO_4, similarly as for the enthalpy of salting the minimum was not reached. The values of apparent molar volumes *vs.* salt concentration at 48 h after the dissolution of the bovine albumin in the buffer were also determined. In this case the course of $V_{\phi,3}=f(m)$ function differs from those determined 1 h after the dissolution of the bovine albumin in the buffer. Although the minimum of apparent molar volume occurs in similar concentrations (Figs 1–3), but the change of apparent molar volume *vs.* concentration are not the same.

The values obtained for ΔH and $V_{\phi,3}$ for NaCl and $(NH_4)_2SO_4$ clearly point to a region in which, under increasing salt concentration, there is a change from salting-in to salting-out process. On the basis of the obtained minima of the respective functions it is possible to determine the ability of the salts studied to precipitate proteins. The data obtained for Li_2SO_4 point to another course of salting process and will be a subject of further investigations.

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