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CALORIMETRIC AND VOLUMETRIC DATA OF SALTING OF ALBUMIN FROM HUMAN SERUM USING NaCl SOLUTIONS

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Apparent molar volume and enthalpy changes for mixing NaCl (aq.) with albumin from human serum (aq.) are experimentally determined (25°C). Calorimetric experiments were carried out in an LKB 10700-2 calorimeter, whereas volumetric measurements were realized using an Anton Paar 60/602 densimeter. The density measurements were made after 1 and 24 h of the dissolution in the buffer (pH 4.2). The relation between the changes of the enthalpy and apparent molar volumes *vs.* molality of NaCl were determined. The obtained data are discussed together with data obtained previously for bovine albumin and hen egg lysozyme solutions with NaCl, Li_2SO_4 and $(NH_4)_2SO_4$ salts of various concentration. As results correlations between the changes of enthalpy of salting and apparent molar volumes vs. molality of salts were made.

Keywords: *albumin from human serum, calorimetry, density, NaCl, salting processes*

Introduction

Salts are often used to modify the conformational stability and solubility of biological macromolecules. Salts with various ions are often used in study of the precipitation and crystallization of proteins. Much information has been gathered about the mechanism of precipitation and crystallization of proteins [1], but quantitative thermodynamic parameters that control the crystallization of proteins were rarely used. This is true especially of calorimetric and volumetric investigations.

In 1997 we reported [2] calorimetric and small-angle light scattering and calorimetric investigations on the kinetics of precipitation of lysozyme in the presence of NaCl at various concentrations. Earlier calorimetric and kinetic studies include those by Tarizawa and Hayashi [3], Shall *et al.* [4], Sibille and Pusey [5], and Darcy and Wiencek [6].

Enthalpy and apparent molar volumes changes for addition of Li_2SO_4, $MgCl_2$, NaCl and $(NH_4)_2SO_4$ to lysozyme [7–10] and for addition of NaCl, Li_2SO_4 and $(NH_4)_2SO_4$ to bovine albumin were also the subject of our previous determination [11]. From the experimental data obtained it was stated, that: a) the salting process is connected with negative enthalpy changes; b) an increase of salt concentration increases the negative value of enthalpy changes; c) the course of changes of the enthalpy with concentration of salts is not monotonic. There exist the regions, in which some deviations are observed from the regular course of enthalpy changes. Determined experimentally the course of changes of apparent molar volumes $V_{\phi,3}$ of proteins studied *vs.* salts concentration follows a similar pattern as the enthalpy changes. For example, in the case of lysozyme salting by sodium chloride the apparent molar volume $V_{\phi,3}$ over the concentration range 0.3 mol kg^{-1} NaCl [10] decreased in relation to the apparent molar volume value of buffer lysozyme solution by $\Delta V_{\phi,3}$= $2.98 \cdot 10^2$ cm^3 mol^{-1} and at this concentration $m_{\min}$ of the salt $\Delta V_{\phi,3}$ attains a minimum. Starting with the concentration of 0.3 mol kg^{-1} NaCl apparent molar volume begins to rise to achieve a maximum value of $V_{\phi,3}$=$108 \cdot 10^2$ cm^3 mol^{-1} at concentration $m_{\max}$= 0.68 mol kg^{-1} NaCl. This kind of changes in the course of the apparent molar volumes as well as enthalpy changes was also observed in the case of lysozyme salting by other salts (i.e. ammonium sulfate and lithium sulfate) [9] as well as in the case of bovine albumin salting [11].

As it results from the performed investigations, in the concentration range 0–0.64 mol kg^{-1} there occurs bimodal variation of the enthalpy and apparent molar volumes values. A similar variation was observed by Retailleau [12] on the curves of solubility of lysozyme at various concentration of NaCl over a large pH range: a first step decrease up to 0.2–0.3 mol kg^{-1} NaCl and then a moderate decrease at higher salt concentration up to 0.6 mol kg^{-1} NaCl. These changes proceed in the process of binding of ions on the protein surface in the salting process. Retailleau is of the opinion that their occurrence is caused by the decreasing disturbance of solvation shell. When concentration exceeds $m_{\max}$ the ions start to be ejected from solvation shell and at $m_{\max} \cong 0.6$ mol kg^{-1} NaCl aggregation process begins. The occurrence of the process of binding of ions on the

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binding sites on lysozyme surface within the concentration range 0–0.64 mol kg^{-1} NaCl was the subject of investigations realized using titration calorimetry [7, 8], conduction calorimetry as well dynamic light scattering methods [10].

In previous papers [9–11] a fairly good agreement was found of the enthalpy and apparent molar volumes changes *vs.* salts concentration in the concentration range m_{min}–m_{max}. The main purpose of the present paper is to determine a correlation between the changes of the enthalpy and apparent molar volumes as well as concentration range m_{min}–m_{max} of salts added, in which the salting process preceding aggregation occurs. Previously obtained calorimetric and volumetric data were additionally enriched by experimentally determined data of apparent molar volumes and enthalpy change values of the albumin from human serum *vs.* NaCl concentration.

Experimental

Materials and methods

Crystallized and lyophilized albumin from human serum (molar masses 66478) was purchased from Sigma (A9511-5G). Suprapure sodium chloride was obtained from Merck (catalogue number 106406). All experiments were performed in the buffer containing 0.1 M sodium acetate (pH 4.2) at 25°C, prepared with distilled water, degassed and deionized.

Calorimetric experiments were carried out in an LKB 10700-2 batch calorimeter. The calorimeter was tested by the Joule's effects generated after each calorimetric measurement. Equal volumes (2 cm^3) of the albumin from human serum in the buffer were mixed with NaCl buffered solutions. The experiments were carried out at different concentration of salt and constant concentration of albumin from human serum.

The measurement of mixing of buffer salt solution into the buffered protein solution (or vice versa) was not enough to determine the enthalpy of salting. This heat effect is the result of superposition of all individual changes occurring in the system. 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 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 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 salt in the buffer and Q_3 is heat of dilution of albumin from human serum in the buffer.

The heat of dilution Q_2 of sodium chloride solution in the buffer and heat of dilution Q_3 of albumin from human serum in the buffer were determined experimentally.

Densities were measured with an Anton Paar 60/602 digital densimeter thermostated to within ±0.002°C. Densities were determined 1 and 24 h after albumin from human serum had been dissolved in the buffer solution containing the salt. The uncertainty in measurements, $\Delta V_{\phi,3}$ was about ±0.5 cm^3 mol^{-1}, whereas the uncertainty in molality determinations, Δm, was $\pm 1 \cdot 10^{-5}$ m.

Results and discussion

The obtained calorimetric data Q_1, Q_2, and the values of final concentrations of albumin from human serum m^f_{alb} and electrolyte m^f_{elect} solutions in the buffer as well as masses of protein were collected in Table 1. Experimentally determined heat of dilution of NaCl in the buffer, Q_2, *vs.* molality was expressed by the relation:

Table 1 Results of calorimetric investigations (albumin from human serum)[a]

m^f_{alb}/mol kg^{-1}	m_{alb}/g	m^f_{NaCl}/mol kg^{-1}	Q_1/J	Q_2/J
0.001199	0.29401	0.21981	0.255	–0.121
0.001207	0.29622	0.33778	0.172	–0.227
0.001225	0.30150	0.44402	0.032	–0.469
0.001215	0.29717	0.57029	–0.171	–0.746
0.001231	0.29899	0.67872	–0.442	–1.020
0.001229	0.29599	0.74418	–0.582	–1.201
0.001224	0.29431	0.81112	–0.713	–1.393
0.001232	0.29794	0.85725	–0.878	–1.550
0.001243	0.29918	0.92068	–1.057	–1.744
0.001243	0.30058	1.05842	–1.437	–2.228
0.001222	0.29034	1.20396	–1.810	–2.715
0.001237	0.29444	1.32238	–2.164	–3.158

[a]Key: m^f_{alb} and m^f_{NaCl} denote the final concentrations of albumin and NaCl; Q_1 and Q_2 – the total heat effect and heat of dilution of NaCl, respectively; 0.1 M sodium acetate buffer, pH 4.2

Q_2 (J g^{-1})=2.688 (m^f_{elect})^2–12.181m^f_{elect} (r=0.994)

As can be seen from the values collected in Table 1 the contribution of the heat of dilution Q_2 values in the total heat effects Q_1 is considerable. The heat effect Q_3 is constant and characterized by small values (0.041 J) in comparison with the Q_1 values.

The densities measured after 1 and 24 h (after dissolution of the albumin in the buffer) and the apparent molar volumes calculated from the density data are given in Table 2, where m_2 and m_3 are molality of NaCl and molality of protein, d_3 is the density of a protein–buffer–electrolyte solution, d_2 is the density of the buffer–electrolyte solution and $V_{\phi,3}$ is the apparent molar volume of albumin from human serum. The values of apparent molar volume given in Table 2, when m_2=0, determined after 1 h, are on average 516.48·10^2 cm^3 mol^{-1} and after 24 h they are 507.34·10^2 cm^3 mol^{-1}.

Figure 1 presents the changes of enthalpy, ΔH, calculated in kJ mol^{-1} of albumin from human serum as depending on the concentration of NaCl, whereas Fig. 2 shows the changes in apparent molar volume, $\Delta V_{\phi,3}$, within the same concentration range, calculated as a difference between the apparent molar volume of albumin from human serum at a given NaCl concentration and the value of the apparent molar volume of albumin from human serum in buffered solution devoid of electrolyte, then m_2=0. In the concentration range 0–0.4 mol kg^{-1} of NaCl there occurs both a decrease of apparent molar volume $V_{\phi,3}$ by 8.9·10^2 cm^3 mol^{-1} and a decrease of enthalpy value by 101.3 kJ mol^{-1}. After passing a minimum at NaCl concentration 0.4 mol kg^{-1} the $V_{\phi,3}$ value increases (Fig. 2) up to the concentration of 0.6 mol kg^{-1} NaCl, to which corresponds an increase of $V_{\phi,3}$ value by 3.5·10^2 cm^3 mol^{-1}. On the curve ΔH=f(m) (Fig. 1) one can observe in this concentration range a small inflexion which is related to the enthalpy increase by 17.9 kJ mol^{-1}. The concentration range δ_m=m_{min}–m_{max}, equal to about 0.2 mol kg^{-1}, corresponds to the changes observed between a minimum and a maximum on the $\Delta V_{\phi,3}$ curve. It should be emphasized here that the difference δm corresponding to this process assumes different values depending on the

Table 2 Densities and apparent molar volumes of albumin from human serum at various NaCl concentrations after 1 and 24 h (25°C)[a]

Time/h	m_2/mol kg^{-1}	m_3/mol kg^{-1}	d_2/g cm^{-3}	d_3/g cm^{-3}	$V_{\phi,3}$·10^2/cm^3 mol^{-1}
1	0	0.001099	0.99887	1.01451	516.82
24			0.99887	1.01562	507.26
1	0	0.001103	0.99887	1.01490	516.15
24			0.99887	1.01565	507.51
1	0.20354	0.001103	1.00702	1.02262	512.74
24			1.00722	1.02320	509.12
1	0.30495	0.001103	1.01108	1.02691	509.13
24			1.01113	1.02700	508.74
1	0.40365	0.001107	1.01505	1.03092	507.87
24			1.01515	1.03113	506.73
1	0.50030	0.001100	1.01849	1.03383	510.56
24			1.01889	1.03400	509.52
1	0.60813	0.001102	1.02298	1.03808	511.38
24			1.02241	1.03791	507.97
1	0.70511	0.001100	1.02638	1.04157	509.02
24			1.02663	1.04250	502.87
1	0.81110	0.001075	1.03009	1.04533	504.38
24			1.03015	1.04580	500.55
1	0.90768	0.001114	1.03434	1.05012	502.65
24			1.03409	1.04973	499.42
1	1.00626	0.001105	1.03775	1.05320	503.25
24			1.03820	1.05236	500.05
1	1.11353	0.001103	1.04190	1.05616	511.89
24			1.04143	1.05651	502.49
1	1.20507	0.001107	1.04518	1.05899	515.06
24			1.04542	1.05987	509.42
1	1.30398	0.001102	1.04848	1.06291	507.94
24			1.04895	1.06331	506.01

[a]Key: m_2 – molality of NaCl; m_3 – molality of albumin; d_2 – density of binary NaCl – buffer solution; d_3 – density of ternary albumin – NaCl – buffer solution; $V_{\phi,3}$ – apparent molar volume of albumin

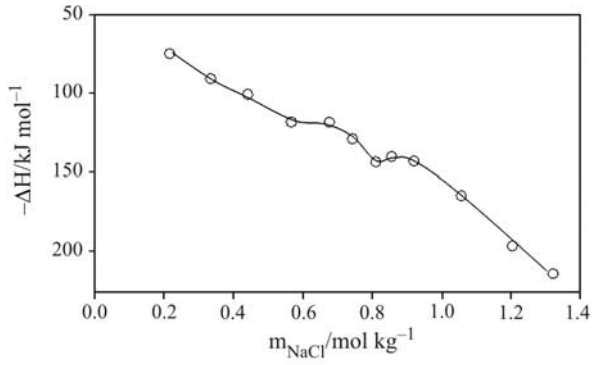

Fig. 1 Enthalpy changes of albumin from human serum ΔH in relation to m sodium chloride

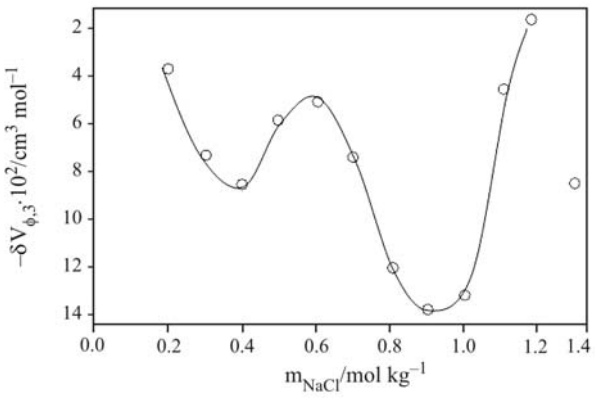

Fig. 2 The changes of $\Delta V_{\phi,3}$ values of albumin from human serum *vs.* concentration m NaCl

kind of protein (albumin from human serum, bovine albumin, or hen egg lysozyme) and salt. This is well seen from the data in Table 3. The data were obtained in the same laboratory, by using the same measurement devices and the same methods of measurement and calculation. In Table 3 are collected concentration values m_{min} and m_{max}, the corresponding to them enthalpy values ΔH_{min}, ΔH_{max}, and the apparent molar volume values $V_{\phi,3min}$ and $V_{\phi,3max}$. From the given data it results that the changes $\delta\Delta H=\Delta H_{max}-\Delta H_{min}$ and $\delta V_{\phi,3}=V_{\phi,3min}-V_{\phi,3max}$ depend strictly both on the concentration values as well as on $\delta m=m_{max}-m_{min}$ increment. The changes are the smallest in the case of the presence of electrolytes in which the process of ions bonding on the protein surface (salting process) occurs within the least δm concentration range (e.g. lysozyme in the presence of $(NH_4)_2SO_4$), and the greatest in the case of electrolytes causing the process of ions bonding on the protein surface in a considerably wide range of concentrations (e.g. bovine albumin in the presence of NaCl (Table 3)). Moreover, it does not matter if this process begins at a higher or lower concentration. For instance, lysozyme salting by NaCl begins at low electrolyte concentration of 0.31 mol kg^{-1} of and finishes already at electrolyte concentration of 0.68 mol kg^{-1}, similarly as salting of albumin from human serum (0.44 and 0.68 mol kg^{-1}). On the other hand, salting of bovine albumin begins at a similar concentration of 0.41 mol kg^{-1}, but it finishes at a concentration of 1.06 mol kg^{-1} of NaCl. This increased δm concentration range in which the salting process occurs, results in a considerable decrease of the value of enthalpy of salting and also in changes of the apparent molar volume of bovine albumin (Table 3).

Table 3 Comparison of the enthalpies and apparent molar volumes of proteins in the buffer with salts

	m_{min}	m_{max}	δm	ΔH_{min}	ΔH_{max}	$\delta\Delta H$	$V_{\phi,3min}\cdot10^2$	$V_{\phi,3max}\cdot10^2$	$\delta V_{\phi,3}\cdot10^2$
1 albumin (b)*	0.41	1.06	0.65	–105.9	–193.5	–87.6			
NaCl	0.41	1.00	0.59				–5.9	0.8	6.7
2 albumin (h)	0.44	0.68	0.24	–101.3	–119.2	–17.9			
NaCl	0.40	0.61	0.21				–8.6	–5.1	3.5
3 albumin (b)*	0.19	0.93	0.74	–48.7	6.3	55.0			
Li_2SO_4	0.40	1.10	0.70				0.2	19.2	19.0
4 albumin (b)*	0.34	0.96	0.62	–45.8	–67.0	–21.2			
$(NH_4)_2SO_4$	0.60	0.90	0.30				–0.5	4.5	5.0
5 lysozyme (e)*	0.31	0.68	0.38	–22.8	–44.5	–21.7			
NaCl	0.30	0.68	0.38				–2.9	1.0	3.9
6 lysozyme (e)*	0.13	0.24	0.11	–5.9	–9.0	–3.1			
Li_2SO_4	0.05	0.20	0.15	3.0			–1.3	2.3	3.6
7 lysozyme (e)*	1.00	1.10	0.10	–76.5	–54.1	22.5			
$(NH_4)_2SO_4$	1.00	1.10	0.10				–2.9	0.1	3.0
8 lysozyme (e)*	0.32	0.76	0.44	4.5	20.1	15.6			
$MgCl_2$	0.30	0.76	0.46				–3.0	3.7	6.7

(b) bovine, (h) from human, (e) hen egg; m_{min}, m_{max} – molalities, δm – molality difference $m_{max}-m_{min}$ (mol kg^{-1}); ΔH_{min}, ΔH_{max} and $\delta\Delta H$ – minimum and maximum enthalpy of proteins and enthalpy difference $\Delta H_{max}-\Delta H_{min}$ (kJ mol^{-1}); $V_{\phi,3min}$, $V_{\phi,3max}$ and $\delta V_{\phi,3}$ – apparent molar volumes and difference $V_{\phi,3max}-V_{\phi,3min}$ (cm^3 mol^{-1}); *values of ΔH and $V_{\phi,3}$ calculated from data in [9–11]

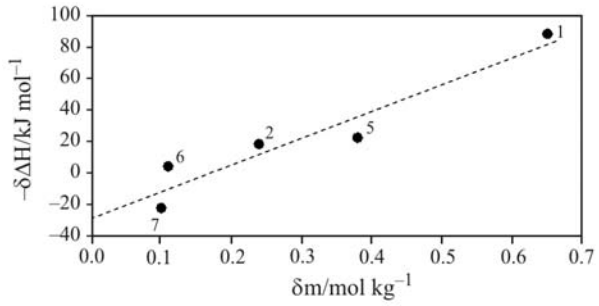

Fig. 3 The changes in the increment of the enthalpy of salting $\delta\Delta H$ *vs.* $\delta m=m_{min}-m_{max}$ No. 1–7, Table 3

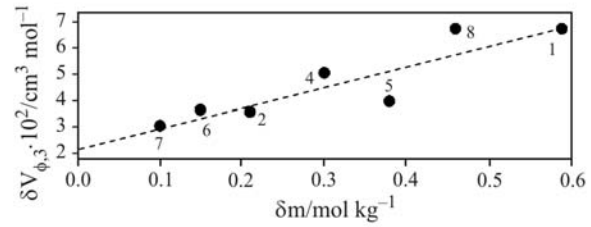

Fig. 4 The changes in the increment of $\delta V_{\phi,3}$ *vs.* $\delta m=m_{min}-m_{max}$. No. 1–8, Table 3

Conclusions

Figures 3 and 4 present diagrams of the changes of enthalpy, $\delta\Delta H$, and apparent molar volume, $\delta V_{\phi,3}$ *vs.* concentration range, δm, as related to the investigated process. It can be said that the changes of enthalpy and apparent molar volumes are quantitatively related with the concentration range δm of electrolyte for which salting process occurs: the higher the concentration range is, the more pronounced are the changes of the enthalpy and apparent molar volumes. Even the δm increase by 0.1 mol kg^{-1} causes an increase of both $\delta\Delta H\cong 17$ kJ mol^{-1} and $\delta V_{\phi,3}$ by about $0.8\cdot10^2$ cm^3 mol^{-1}. The only exception to this rule is exhibited by bovine albumin in the presence of Li_2SO_4 in the case when the positive value of $\delta\Delta H$ is disproportionately high (55.0 kJ mol^{-1}), similarly as the value of $\delta V_{\phi,3}=19.0$ cm^3 mol^{-1}. The latter value is definitely different from other $\delta V_{\phi,3}$ values, ranging from 3.0 to $6.7\cdot10^2$ cm^3 mol^{-1} (Table 3).

It is also worth noting that the changes of apparent molar volumes after 24 h from dissolution of the albumin from human serum in the buffer with NaCl are much lower from those determined after 1 h. In the solution left for 24 h the salting process proceeds within a lower concentration range ($\delta m\cong 0.1$ mol kg^{-1}) than in the case of the solution whose density was measured after 1 h (Table 3). This is evident, taking into account the fact that the process of salting proceeds continuously at a given electrolyte concentration, until the process is finished.

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