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CALORIMETRIC AND VOLUMETRIC DATA OF SALTING OF HEN EGG LYSOZYME USING NaCl SOLUTION AT pH 8.8

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Heat effects and densities of hen egg lysozyme in the phosphate buffer at pH 8.8 and various NaCl concentrations were determined at 25°C by LKB 10700-2 microcalorimeter and an Anton Paar 60/602 densimeter. The relation between the changes of the enthalpy and apparent molar volumes *vs*. molality of NaCl were determined. The data are discussed together with the data obtained previously for hen egg lysozyme solutions with NaCl salt in Na-acetate buffer pH 4.2.

Keywords: *calorimetry, density, lysozyme, NaCl solutions, salting processes*

Introduction

The study of the complexation process of simple proteins with mono- and divalent salt ions was the subject of a number of our works [1–4]. Various physicochemical methods were used including calorimetry, dynamic light scattering and densimetry. Particular attention was given to measurements of heat effects produced as a result of the addition of various concentrations of salts. Simultaneously with these studies apparent molar volumes were densimetrically determined. It was shown that while salt is being added to protein, changes in the enthalpy are negative and as the salt concentration increases the negative value of the enthalpy increases, too. In certain regions of salt concentration there occur material changes in the course of curve of enthalpy function plotted as a function of concentration of the added salt. Moreover, changes in the apparent molar volumes [5–9] as well as solubility [10] have a similar course in the function of salt concentration. These observations concerned buffered solutions (pH 4.2) of hen egg lysozyme, bovine albumin, albumin from human serum upon the addition of salts: sodium chloride and magnesium chloride and sulfates: lithium and ammonium. On the basis of the obtained data several conclusions have been drawn concerning the process of protein precipitation.

The aim of the present work was to determine whether in solutions with pH 8.8 one can observe a similar nature of changes in enthalpy and apparent molar volumes in the function of salt concentration and come to similar conclusions. The object of the studies were solutions of hen egg lysozyme with various concentrations of NaCl.

Experimental

Hen egg lysozyme was purchased from Fluka (62970), suprapure sodium chloride was obtained from Merck (106406). All experiments were performed in the phosphate buffer (pH 8.8) 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 lysozyme in the buffer were mixed with NaCl buffered solutions. The experiments were carried out at different concentrations of salt (0.10–1.51 mol kg^{-1}) and constant concentration (0.003 mol kg^{-1}) of lysozyme.

The heat of salting Q was calculated as equal to $Q=Q_1-Q_2-Q_3$ where Q_1 is the total heat effect measured in the calorimeter, Q_2 is the heat of dilution of salt in the buffer and Q_3 is the heat of dilution of lysozyme in the buffer.

The heat of dilution Q_2 of sodium chloride solution in the buffer and heat of dilution Q_3 of lysozyme in the buffer were determined experimentally.

Densities were measured with an Anton Paar 60/602 digital densimeter thermostated within ±0.002°C. Densities were determined 1 h after lysozyme had been dissolved in the buffer solution containing the salt. The uncertainty in measurements, $\Delta V_{\phi,3}$, was ±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, Q_3 and the values of final concentrations of lysozyme m^{f}_{lys} and

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m^{f}_{NaCl} electrolyte solutions in the buffer as well as masses of protein were collected in Table 1. The experimentally determined data of heat of dilution of NaCl in the buffer, Q_2, were collected in Table 2.

The densities and the apparent molar volumes calculated from the density data are given in Table 3, 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 lysozyme.

Figure 1 presents the changes of enthalpy, ΔH and the changes of apparent molar volume $V_{\phi,3}$ of lysozyme as depending on the concentration of NaCl. The apparent molar volumes data are presented in the form of the differences $V_{\phi,3}$ between the apparent molar volume of lysozyme at a given NaCl concentration and the value of the apparent molar volume of lysozyme in the buffered solution devoid of electrolyte (m_2=0, $V_{\phi,3}$=106.88·10^2 cm^3 mol^{-1}).

While the sodium chloride concentration is increasing in the initial period of the salting process, the enthalpy value shows a linear decrease (Fig. 1). For the concentration m_{min}=0.2 mol kg^{-1} NaCl a characteristic deflexion can be seen on the curve. The enthalpy has it maximum for the concentration m_{max}= 0.75 mol kg^{-1} NaCl. On the curve of dependence of the apparent molar volume on NaCl concentration a clear deflexion is also noticeable at the concentration 0.2 mol kg^{-1}. From this concentration the value of apparent molar volume is still minimally decreasing to the concentration of approx. 0.4 mol kg^{-1} NaCl. The maximum of apparent molar volume occurs, however, at a slightly lower concentration than in the case of the enthalpy of the salting process as in this case the concentration equals 0.65 mol kg^{-1} NaCl.

Table 1 Results of calorimetric investigation (pH 8.8, 25°C)

m^{f}_{NaCl} /mol kg^{-1}	m^{f}_{lys} /mol kg^{-1}	Mass lys/g	Q_1/J	Q_2/J	Q_3/J	Q_1–Q_2–Q_3/kJ mol^{-1}
0	0.001571	0.08733			–0.056	
0.05197	0.001536	0.08562	–0.0146	0.0054	–0.056	6.013
0.10618	0.001546	0.08560	–0.0200	–0.0177	–0.056	8.970
0.15884	0.001512	0.08539	–0.0435	–0.0590	–0.056	11.974
0.21021	0.001578	0.08754	–0.0775	–0.1144	–0.056	15.175
0.25971	0.001599	0.08845	–0.1241	–0.1783	–0.056	17.816
0.31706	0.001556	0.08660	–0.2059	–0.2731	–0.056	20.343
0.37797	0.001534	0.08671	–0.2780	–0.3663	–0.056	23.955
0.48030	0.001560	0.08621	–0.4617	–0.5662	–0.056	26.622
0.59250	0.001566	0.08494	–0.7139	–0.8293	–0.056	28.854
0.67514	0.001575	0.08578	–0.8900	–1.0580	–0.056	37.342
0.75478	0.001545	0.08614	–1.0374	–1.3060	–0.056	53.882
0.82246	0.001581	0.08514	–1.2854	–1.4839	–0.056	42.745

Table 2 Heat of dilution NaCl in the buffer

Initial concentration/mol kg^{-1}	Mass NaCl/g	Final concentration/mol kg^{-1}	Heat of dilution/	
			J	kJ mol^{-1}
0.10100	0.01165	0.05036	0.0054	0.0270
0.20613	0.02407	0.10375	–0.0177	–0.0429
0.30074	0.03485	0.15068	–0.0590	–0.0990
0.41304	0.04790	0.20974	–0.1144	–0.1396
0.50734	0.05927	0.25860	–0.1783	–0.1758
0.60557	0.07125	0.31255	–0.2731	–0.2239
0.70251	0.08412	0.36310	–0.3663	–0.2540
0.90456	0.10739	0.47615	–0.5662	–0.3080
1.10352	0.13248	0.60095	–0.8293	–0.3650
1.25040	0.15209	0.66043	–1.0580	–0.4065
1.36319	0.16373	0.72404	–1.1809	–0.4210
1.50610	0.18298	0.81461	–1.4839	–0.4739

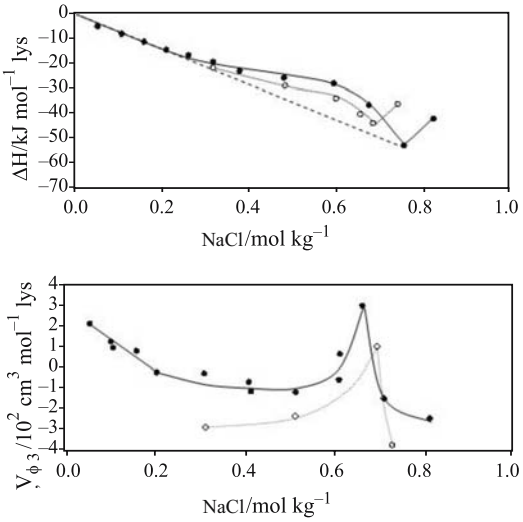

Fig. 1 Comparison of the enthalpy and apparent molar volume data of lysozyme at various NaCl concentration • – pH 8.8, ○ – pH 4.2 [6]

The comparison of these curves to the curves determined previously [6] for solutions of hen egg lysozyme in acetate buffer, pH 4.2, points out to the same nature of their course (Fig. 1). The values are, however, different ΔH, $V_{\phi,3}$ as well as the range of the concentrations $\delta m=m_{min}-m_{max}$ occurring between the deflexion on the curve and the maximum of the value. In the case of the lysozyme in a solution of pH 8.8 the maximum of the enthalpy amounts to –53.9 kJ mol^{-1} whereas in a solution of pH 4.2 its value is –44.5 kJ mol^{-1}. The value of the apparent molar volume in the concentration m_{max} equals 2.94 mol kg^{-1} (pH 8.8) and 1.0 cm^3 mol^{-1} (pH 4.2) [7].

In our consideration it was assumed that the maximum value of the enthalpy and the value of apparent molar volume are connected with the quotient of the NaCl concentration to the lysozyme concentration. In case of a solution of pH 8.8 this relation equals 488, whereas in case of the solution of pH 4.2 it equals 440. For other proteins (bovine albumin and albumin from human serum) examined in NaCl solutions of pH 4.2 [5–7] the value of the enthalpy ΔH_{max} is also proportional to the quotient of the electrolyte concentration and the protein concentration (Table 4).

In this work changes in the enthalpy $\delta\Delta H$ and apparent molar volume $\delta V_{\phi,3}$ between concentrations m_{max} and m_{min} equal 38.7 kJ mol^{-1} and 4.2·10^2 cm^3 mol^{-1}. In a solution of pH 4.2 these values equal 21.7 kJ mol^{-1} and 3.9·10^2 cm^3 mol^{-1}, respectively [6]. These differences in values of $\delta\Delta H$ and $\delta V_{\phi,3}$ are connected with the range of concentrations δm, in which the salting process is running, assuming that this process begins at concentration m_{min} and ends at concentration m_{max}. At concentration exceeding m_{max} the aggregation of protein begins. In the discussed case (pH 8.8) δm=0.75–0.2= 0.55 mol kg^{-1} ($\delta\Delta H$); δm=0.65–0.4=0.25 mol kg^{-1} ($\delta V_{\phi,3}$) and (pH 4.2) δm=0.68–0.31=0.37 mol kg^{-1} ($\delta\Delta H$); δm=0.68–0.30=0.38 mol kg^{-1} ($\delta V_{\phi,3}$) [7].

Previously [7] it was indicated on the example of lysozyme, bovine albumin and albumin from human serum, examined in solutions of NaCl, $MgCl_2$, $(NH_4)_2SO_4$, Li_2SO_4, that the values of δm are directly proportional to the difference of the values $\delta\Delta H=\Delta H_{max}-\Delta H_{min}$ and $\delta V_{\phi,3}=V_{\phi,3max}-V_{\phi,3min}$, which are related to the beginning and ending of the salting process. These investigations were conducted in solutions with pH 4.2. This work concerning the study of lysozyme in the solution of

Table 3 Density and apparent molar volumes of lysozyme at various NaCl concentration

m_{2NaCl}/mol kg^{-1}	m_{3lys}/mol kg^{-1}	d_2/g cm^{-3}	d_3/g cm^{-3}	$V_{\phi,3}\cdot10^2$/cm^3 mol^{-1}
0	0.001571	1.006255	1.011294	106.88
0.05370	0.001531	1.008353	1.013386	108.94
0.10153	0.001535	1.010582	1.015737	108.06
0.10478	0.001500	1.011193	1.016268	107.78
0.15738	0.001515	1.012249	1.017388	107.62
0.20136	0.001519	1.014211	1.019496	106.61
0.30723	0.001516	1.018378	1.023615	106.56
0.40416	0.001521	1.022599	1.027874	106.12
0.40874	0.001534	1.022242	1.027635	105.68
0.50798	0.001510	1.026576	1.031846	105.63
0.60292	0.001545	1.029723	1.033334	106.22
0.60490	0.001540	1.029900	1.034941	107.46
0.65439	0.001534	1.032574	1.037182	109.82
0.70211	0.001522	1.033581	1.038862	105.33
0.80298	0.001509	1.042594	1.037240	104.34

Table 4 Comparison of the enthalpy ΔH_{max} at various m_{NaCl}/m_{prot}

	ΔH_{max}/kJ mol^{-1}	m_{NaCl}/m_{prot}
Hen egg lysozyme (pH 4.2)	–44.5 [6]	440
Hen egg lysozyme (pH 8.8)	–53.8	488
Albumin from human (pH 4.2)	–119.2 [7]	551
Bovine albumin (pH 4.2)	–193.5 [7]	886

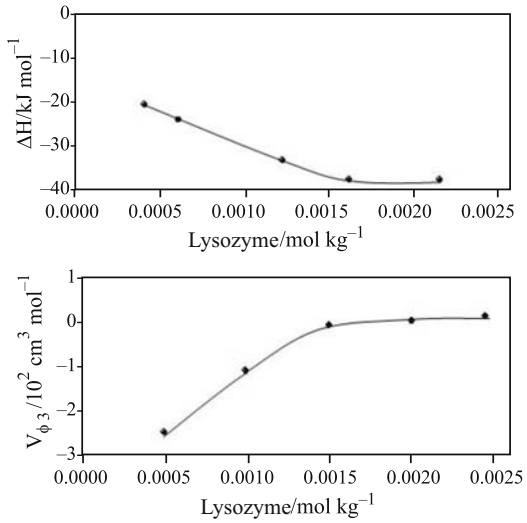

Fig. 2 Comparision of the enthalpy changes ΔH and changes in apparent molar volume $V_{\phi,3}$ in various lysozyme concentration at a fixed NaCl concentration. Data on ΔH from [6], data on $V_{\phi,3}$ from [8]

NaCl of pH 8.8 confirms this regularity, regardless of the fact that in this case the salting process begins at a slightly lower concentration and runs to a concentration higher than in the case of solutions with pH 4.2. It is consistent with the observations of Retailleau *et al*. [10] concerning the solubility of lysozyme in solutions with various pH in the presence of NaCl, in which it was shown that solubility of lysozyme depends on pH of the solution, especially in the first period of the salting process, and changes at certain characteristic concentrations of NaCl, i.e. at 0.3 mol kg^{-1} NaCl and pH 4.3 the solubility curve is clearly bent, whereas at pH 8.4 this bend occurs earlier, namely already at NaCl concentration equal 0.15 mol kg^{-1}.

The obtained values of enthalpy ΔH_{max} and apparent molar volume $V_{\phi,3max}$ relate to the specified concentration of lysozyme 0.0015 mol kg^{-1}. At lysozyme concentrations m<0.0015 mol kg^{-1} and concentration m_{max} NaCl this maximum is not achieved. It can be seen in Fig. 2, which shows the dependencies of enthalpy of the salting process and apparent molar volumes on lysozyme concentration at constant concentration of NaCl approx. 0.7 mol kg^{-1} (pH 4.2) [6–8]. Values of enthalpy of the salting process ΔH at lysozyme concentration from 0.0004 to 0.0015 mol kg^{-1} change by up to 17 kJ mol^{-1} [6], whereas values of apparent molar volume of lysozyme $V_{\phi,3}$ within the similar concentration range change by $2.5 \cdot 10^2$ cm^3 mol^{-1} [8]. Only at lysozyme concentration of 0.0015 mol kg^{-1} values ΔH and $V_{\phi,3}$ achieve a constant value (Fig. 2) corresponding to the maximum on the curves presented in Fig. 1.

It results from the above discussion that the salting process depends on the ratio of electrolyte concentration to protein concentration. The calorimetric and volumetric studies allow to precisely determine the initial and final values of electrolyte concentrations in which the process runs (at a given concentration of protein).

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