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Comparison of enthalpy and apparent molar volume data of salting lysozyme solutions at various NaCl concentrations

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Abstract

Apparent molar volume and enthalpy changes for mixing NaCl (aq.) with lysozyme (aq.) are experimentally determined. The enthalpy change generally becomes more exothermic as the concentration of NaCl increases but exhibits a maximum exothermic effect at 0.68 m NaCl. The apparent molar volume exhibits a minimum at 0.3 m NaCl and a maximum at 0.68 m NaCl with a difference of 3.9×10^2 cm^3 mol^{-1}. The apparent molar volume increment for the process driven by Li_2SO_4, $MgCl_2$ and $(NH_4)_2SO_4$ is similar, 3.4×10^2 cm^3 mol^{-1}.

Keywords: Lysozyme; NaCl solutions; Salting-in; Salting-out; Enthalpy; Apparent molar volume

1. Introduction

Salts are often used to modify the conformational stability and solubility of biological macromolecules. 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 are needed. Calorimetric studies of the effects of added electrolyte on both the kinetics and the enthalpy change for precipitation and crystallization are important. It is also important to establish the conditions for salting-in and salting-out. The former occurs at low concentrations of salt, the latter at higher concentrations where preferential hydration of the ions can desolvate proteins and make them aggregate, precipitate or crystallize [2].

In 1997, we reported [3] calorimetric and small-angle light scattering investigations on the kinetics of precipitation of lysozyme in the presence of NaCl at various concentrations. Earlier calorimetric studies include those by Tarizawa and Hayashi [4], Shall et al. [5], Sibille and Pusey [6], and Darcy and Wiencek [7].

Enthalpy changes for addition of Li_2SO_4, $MgCl_2$ and $(NH_4)_2SO_4$ to lysozyme were also determined by us [8,9]. Studies were also done to compare apparent molar volumes and enthalpy changes. Enthalpy changes and apparent molar volumes vary similarly with concentration of electrolytes [10]. The present work is concerned with hen-egg lysozyme solutions at various NaCl concentrations. The results broaden our knowledge on the enthalpy and apparent molar volume of lysozyme and enable new conclusions about the value of the apparent molar volume in understanding salting processes.

2. Materials and methods

Lyophilized lysozyme was purchased from Sigma and stored at 4 °C. Suprapure sodium chloride was received from Merck. All experiments were performed in a 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 by mixing equal volumes (2 cm^3) of the solutions. Two series of calorimetric experiments were performed. In the first series, the initial concentration of lysozyme solution was kept constant at 0.0030 m and the concentration of NaCl solutions was varied from 0.60 to 1.98 m. In the second series, the concentration of NaCl was kept con-

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stant at 1.31 m and the concentration of lysozyme was varied from 0.0008 to 0.0040 m. The smallest heat quantity measured was 0.001 J.

Densities were measured with an Anton Paar 60/602 digital densimeter. The instrument and the working procedure have been described elsewhere [11]. The uncertainty in measurements, $\delta(V_{\phi,3})$, was about $\pm0.5\ \mathrm{cm^3\,mol^{-1}}$, whereas the uncertainty in molality determinations, $\delta(m)$, was $\pm1\times10^{-6}$ m. The densimeter was thermostated to within ±0.002 °C. Densities were determined 1 h after lysozyme had been dissolved in the buffer solution containing the electrolyte.

3. Results

The calorimetric data from the two series of measurements are given in Tables 1 and 2 in supplementary data files. The final concentrations of lysozyme $m^{\mathrm{f}}_{\mathrm{lys}}$ and electrolyte $m^{\mathrm{f}}_{\mathrm{NaCl}}$ solutions in the buffer and the total heat effects Q_1 measured are given. The heats of dilution of NaCl in the buffer Q_2 and of lysozyme in the buffer Q_3 are given in Table 3 in the supplementary data files. The excess heat effect Q was calculated as $Q = Q_1 - Q_2 - Q_3$.

In the first series of measurements, the contribution Q_2 to the total heat effect Q_1 varied from 18–60%. In the second series, the heat effect Q_2 was practically identical (average value 1.14 J) and its contribution to Q_1 amounted 3–30%.

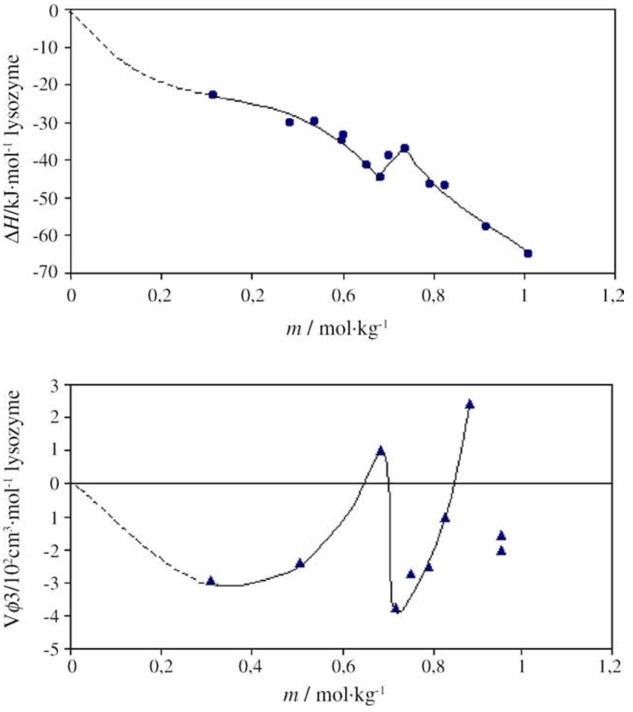

Fig. 1. Comparison of the enthalpy changes for addition of NaCl to lysozyme and apparent molar volume $V_{\phi,3}$ of lysozyme at various NaCl concentrations (Series 1).

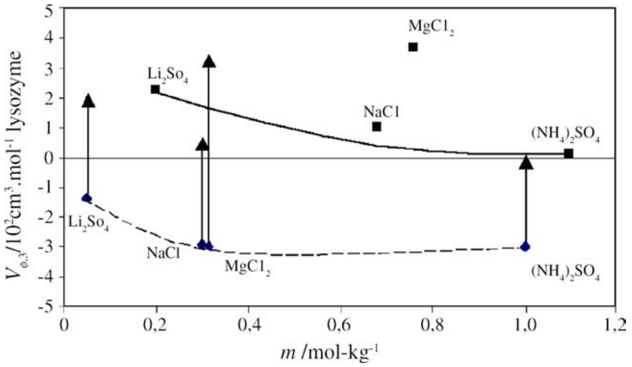

Fig. 2. Apparent molar volume $V_{\phi,3}$ in relation to m with various electrolytes: (♦)$V_{\phi,3\mathrm{min}}$, (■) $V_{\phi,3\mathrm{max}}$. Volume differences, $\Delta V_{\phi,3}$, indicated by arrows (cf. Table 5 in supplementary files).

The heat effect Q_3 was constant in the first series (0.018 J) and in the second series of experiments varied from 0.001 to 0.041 J.

The densities measured and the apparent molar volumes calculated from the density data are given in Table 4 in the supplementary files, where m is the molality of NaCl, d_3 the density of a lysozyme–buffer-electrolyte solution, d_2 the density of a buffer-electrolyte solution and $V_{\phi,3}$ is the apparent molar volume of lysozyme.

The results are presented graphically in Figs. 1 and 2.

4. Discussion

The exothermic enthalpy change for mixing NaCl with lysozyme and the apparent molar volume of lysozyme exhibit maxima at a concentration of 0.68 m NaCl (Fig. 1). This result is consistent with the calorimetric titration data [9] and consistent with Georgalis et al. [12] data measured by the methods of dynamic light scattering (DLS) and optical microscopy. Their maxima in the curves of quasi-stationary radius versus concentration and of the sticking probability on collision versus concentration were found to occur at a concentration of 0.60 m NaCl.

Over the concentration range 0–0.3 m NaCl, the apparent molar volume of lysozyme, calculated as a difference between the values of apparent molar volumes at 0 and 0.3 m, decreased by $2.9\times10^2\ \mathrm{cm^3\,mol^{-1}}$. Starting at 0.3 m NaCl, the apparent molar volume rises to a maximum value of $108.5\times10^2\ \mathrm{cm^3\,mol^{-1}}$ at 0.68 m NaCl. From 0 to 0.68 m NaCl, the enthalpy change increases, to a maximum ($-44.5\ \mathrm{kJ\,mol^{-1}}$).

These data can be interpreted in terms of variation of lysozyme solubility versus NaCl concentration. Retailleau et al. [13] showed that lysozyme solubility decreases steeply up to 0.3 m NaCl and then more slowly at higher ionic strength. In the range 0–0.3 m NaCl salting-in occurs. Sibille and Pusey [6] deduced from the observation of chloride adsorption carried out with fluorescent quencher that the number of bound

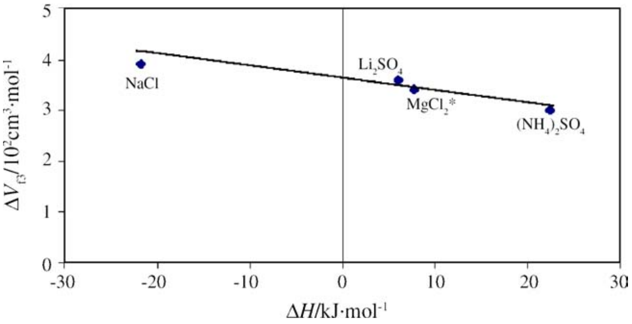

Fig. 3. $\Delta V_{\phi,3}$ vs. ΔH for various electrolytes. (*) $\Delta V_{\phi,3}/2$ and $\Delta H/2$ (cf. Table 5 in supplementary files).

Cl^{-1} ions increases between 0 and 0.35 m NaCl, remains constant up to 0.6 m NaCl and then decreases rapidly above 0.8 m NaCl.

The process occurring in the range 0.3–0.68 m NaCl is delimited by the minimum and maximum values of apparent molar volume. The minimum is the starting point of the process and the maximum indicates completion of this process. The increment in $V_{\phi,3}$ between these points is 3.9×10^2 cm^3 mol^{-1}. This process has been studied with electrolytes other than NaCl, i.e. Li_2SO_4, NaCl, $MgCl_2$ and $(NH_4)_2SO_4$ [10]. These electrolytes give quite similar increments in the apparent molar volume, averaging 3.4×10^2 cm^3 mol^{-1}. As evident from Fig. 2 this phenomenon occurs at different concentrations of the salts, and the enthalpy changes observed within the concentration range $m_{\min}$–$m_{\max}$, are quite different (Fig. 3).

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