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APPARENT MOLAR VOLUMES AND HEAT CAPACITIES OF NUCLEATING LYSOZYME SOLUTIONS AT 25°C

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Abstract

Densities and heat capacities of lysozyme in Na-acetate buffer (pH 4.2) containing 0.64 m sodium chloride at 25°C were determined by Anton Paar 60/602 digital densimeter and differential scanning adiabatic calorimeter DASM-4 in the range of lysozyme concentration 0.000499–0.002450 m. The measurements were made after 1, 24 and 48 h of the dissolution of lysozyme in the buffer. The changes of the values of apparent molar volumes and heat capacities in time were observed.

Keywords: density, heat capacity, nucleating lysozyme solution

Introduction

Lysozyme is a suitable model system for investigating crystallization of biomolecules since it fulfils some important requirements: (i) the protein is stable and its monomeric state is well defined and (ii) aggregation and concomitant crystallization can be easily induced by simple electrolytes, *i.e.,* NaCl upon screening the net positive surface charges.

We have investigated the properties of nucleating lysozyme solutions, at various lysozyme and NaCl concentrations at pH 4.2, by isothermal conduction microcalorimetry and small-angle static light scattering [1]. Pronounced heat-power peaks, that can be attributed to nucleation and growth, appear at definite times, which in turn depend on the supersaturation level. The calorimetric results are in qualitative accordance with the nucleation behavior deduced from small-angle scattering experiments. It was demonstrated, that crystallization of lysozyme in the buffer containing 0.64 m NaCl, appearing after 1–13 h after dissolution of lysozyme in the buffer depend on the small changes of the concentration of lysozyme (of the range 0.5 mmol).

In this paper the lower concentrations of lysozyme (0.5–2.4 mmol kg^{-1}) and the same concentration of sodium chloride (0.64 m) as previously used were investigated. Time dependences of apparent molar volumes and heat capacities of the solution of lysozyme in buffer were investigated.

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Materials and methods

Crystallized lysozyme was purchased from Sigma and dialyzed against water, lyophilized and stored at 4°C in the Institute of Crystallography, Freie Universität, Berlin. All experiments were performed in the buffer containing 0.1 m sodium acetate (pH 4.2) and 0.64 m sodium chloride. Suprapure sodium chloride was purchased from Merck (6406). The solutions were prepared by mass: buffer was added to sodium chloride, obtaining concentration of 0.64 m kg^{-1} buffer, then lysozyme was added. The obtained 5 solutions were stored in 25°C and used for apparent molar volume and heat capacities measurements.

The densities were determined with Anton Paar 60/602 digital densimeter at 25°C. The calibration constant of the densimeter was determined from measurements with dry air and deionized water. The densities were determined after 1, 24 and 48 h after the dissolution of lysozyme in buffer. The apparent molar volumes were calculated from the formula:

$$V_\Phi = M/d - 1000(d - d_0)/mdd_0 \quad (1)$$

where M is the molar mass of the solute (14 300), d and d_0 the density of the solution and solvent respectively and m the molality of the solution. Densities of solvent d_0 (buffer containing 0.64 m sodium chloride) were measured relative to that of pure water ($d_{25°C}$=0.997047 g cm^{-3}). The heat capacity per unit volume of solution was measured relative to that of buffer (containing 0.64 m sodium chloride) using a differential adiabatic scanning microcalorimeter DASM-4 in which the volume of each vessel was equal to 0.47 mL. The instrument and working procedure have been described elsewhere [2]. The calorimeter was calibrated by electric Joule effect and by

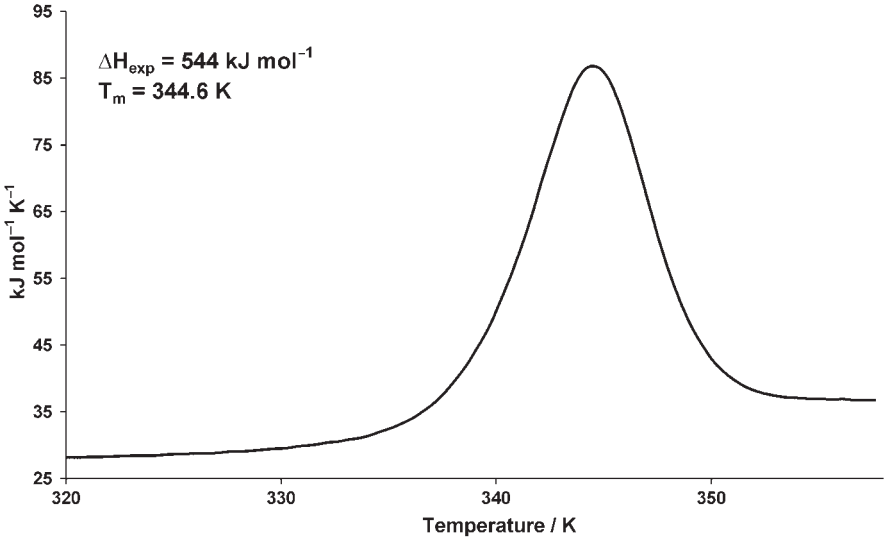

Fig. 1 Temperature dependence of the partial heat capacity of lysozyme

determination of the heat of denaturation of lysozyme (Fig. 1) in the buffer containing 0.64 m sodium chloride at 10– 85°C. Heat of denaturation of lysozyme 544 kJ mol^{-1} is in a good agreement with the value 542 kJ mol^{-1} calculated from the formula: $(\Delta H_d)_{Td}$=17.10+1.5737 T_d (°C) kcal mol^{-1} [3] (1 cal= 4.184 J). The measurements of apparent molar heat capacities of lysozyme were performed at the heating rate of 1°C min^{-1}, at 10–30°C. Similarly as in the case of the apparent molar volumes, the measurements of apparent molar heat capacities after 1, 24 and 48 h after the dissolution of lysozyme in buffer were made.

Results and discussion

The experimental data for the density and apparent molar volumes after 1, 24 and 48 h and the concentration lysozyme *m* of the solutions are collected in Table 1. In V_Φ evaluation the experimentally determined density d_0=1.029260 g cm^{-3} (buffer containing 0.64 m sodium chloride) was used.

Table 1 Density and apparent molar volumes of lysozyme after 1, 24 and 48 h (buffer Na-acetate 0.1 m, pH 4.2, 0.64 m sodium chloride) at 25°C

m	*d*			$V_\Phi \cdot 10^2$		
	1 h	24 h	48 h	1 h	24 h	48 h
0.000499	1.031044	1.031063	1.031192	105.03	104.67	102.22
0.000996	1.032653	1.032723	1.032887	106.43	105.76	104.19
0.001497	1.034160	1.034301	1.034522	107.46	106.57	105.16
0.001999	1.035752	1.035836	1.036067	107.55	107.14	106.04
0.002450	1.037159	1.037205		107.64	107.46	

m – molality: mol kg^{-1}; *d* – density: g cm^{-3}; V_Φ – apparent molar volume: cm^3 mol^{-1} (density of buffer 1.029260 g cm^{-3})

It has been found that the values of the apparent molar volume increase with the increase of lysozyme concentration (Fig. 2a) and change in time. The increase of V_Φ with concentration *m* is non-linear. The highest changes V_Φ=*f(m)* occur for low lysozyme concentrations. With increasing concentration *m* the increase of V_Φ are lower. The lack of experimental data for very low concentrations (to 0.0005 m) makes it impossible to precisely determine the partial molar volume (V_2^0) of lysozyme. For the native lysozyme in water solution this value has been given by Mathatadze *et al.* [4] as V_2^0 (25°C)=$102.80 \cdot 10^2$ cm^3 mol^{-1}. The changes of V_Φ in time are different for the studied lysozyme concentrations. The highest changes are observed for low concentrations (Fig. 2a). For example, the changes in V_Φ in 48 h after dissolution of lysozyme in a buffer are: $2.81 \cdot 10^2$ cm^3 mol^{-1} at a concentration of 0.000499 m and $1.51 \cdot 10^2$ cm^3 mol^{-1} for 0.001999 m (Table 1). In this range of concentrations the number of moles of sodium chloride per one mole of lysozyme decreases four-fold (from 1482 to 302 m) – Fig. 3a.

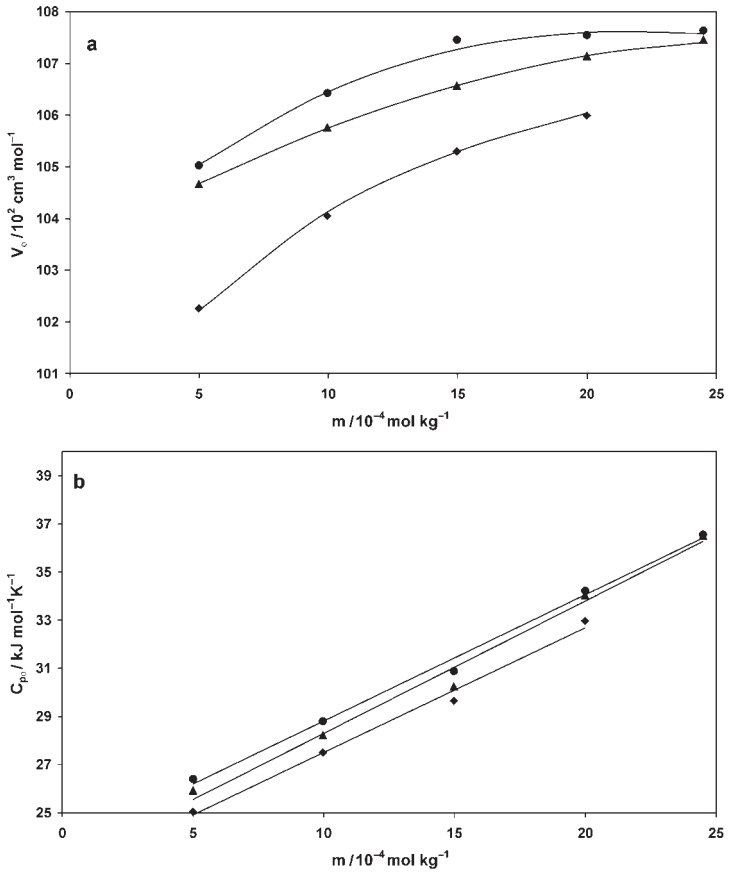

Fig. 2 Apparent molar volume (a) and heat capacity (b) of lysozyme *vs*. molality after 1 h (●), 24 h (▲) and 48 h (♦)

In Table 2 the values of apparent molar heat capacities after 1, 24 and 48 h are presented. The linear relations of $C_{p\Phi}$ on lysozyme concentration m are observed (Fig. 2b):

$$C_{p\Phi} \quad (1\ \text{h}) = 23.57 + 5241\ m\ (\text{kJ mol}^{-1}\ \text{K}^{-1}) \quad r = 0.997 \tag{2}$$

$$C_{p\Phi}\ (24\ \text{h}) = 22.80 + 5496\ m\ (\text{kJ mol}^{-1}\ \text{K}^{-1}) \quad r = 0.994 \tag{3}$$

$$C_{p\Phi}\ (48\ \text{h}) = 22.32 + 5180\ m\ (\text{kJ mol}^{-1}\ \text{K}^{-1}) \quad r = 0.996 \tag{4}$$

Considering the relations (2)–(4) one can conclude that the values of $C_{p\Phi}$ are time dependent. It means the latter experiment was performed the smaller are $C_{p\Phi}$ values. Some dependence concerns the partial molar heat capacities $C^{\circ}_{p,2}$ of lysozyme (23.57±0.36; 22.80±0.54 and 22.32±0.38 kJ mol^{-1} K^{-1}) are linear *vs*. time.

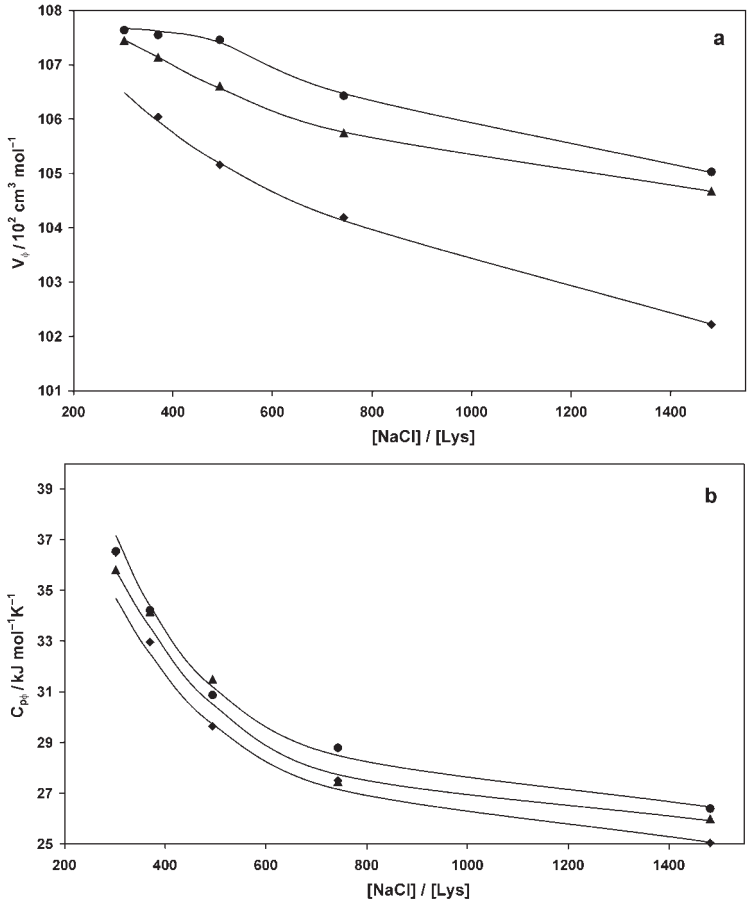

Fig. 3 Apparent molar volume (a) and heat capacity (b) *vs.* molality mol_{NaCl}/ mol_{lys} 1 h (●), 24 h (▲) and 48 h (♦)

Table 2 Apparent molar heat capacities of lysozyme after 1, 24 and 48 h (buffer Na-acetate 0.1 m, pH 4.2, 0.64 m sodium chloride) at 25°C

m	$C_{p\Phi}$		
	1 h	24 h	48 h
0.000499	26.40	25.92	25.04
0.000996	28.80	28.21	27.50
0.001497	30.88	30.24	29.64
0.001999	34.22	34.03	32.96
0.002450	36.55	36.50	

m – molality: mol kg^{-1}; $C_{p\Phi}$ – apparent molar heat capacity: kJ mol^{-1} K^{-1}

Decrease $C^{o}_{p,2}$ of values is related with increase of the relative NaCl concentration (mol_{NaCl}/mol_{lys}) (Fig. 3b).

Conclusions

The lysozyme solutions containing high sodium chloride concentrations undergo changes during time as observed from the differences in the values of apparent molar volumes and heat capacities. The changes of V_{Φ} and $C_{p\Phi}$ depend not only on time but also on sodium chloride concentration suggesting isothermal lysozyme nucleation and growth of nuclei in solution.

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