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Enthalpic pairwise self-association of L-carnitine in aqueous solutions of some alkali halides at $T = 298.15$ K

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ABSTRACT

Knowledge of the influence of ions of various nature on intermolecular hydrophilic and hydrophobic interactions in solutions is required in many research fields. In this paper, dilution enthalpies of zwitterion L-carnitine in aqueous NaCl, KCl and NaBr solutions of various molalities (b = 0 to 3.0 mol · kg^{-1}) have been determined respectively at $T = (298.15 \pm 0.01)$ K and $p = (0.100 \pm 0.005)$ MPa by isothermal titration calorimetry (ITC). In light of the MacMillan–Mayer theory, the 2nd virial enthalpic coefficients (h_2) have been calculated. The h_2 coefficients increase gradually with increasing molality (b) of the three aqueous alkali halides solutions, from small negative values in pure water to relatively larger positive values in solution. The trends of h_2 coefficients are ascribed to the salt effects on the balance between hydrophilic and hydrophobic interactions in pairwise self-associations. It is considered that the size of cations and anions exert influences on h_2 coefficients through their surface charge densities and hydration (or dehydration) abilities.

1. Introduction

Most biomolecules like amino acids, peptides, lipids, *etc.* are amphipathic, that is, they have both hydrophobic and hydrophilic segments in their molecular structures. Knowledge of the influence of ions of neutral salts on hydrophilic and hydrophobic interactions of this kind of biomolecules are of great significance for one to gain insight into some biological problems such as ion channels, protein stability, enzyme activation, protein crystal growth, and (protein + protein) interactions (PPIs) [1–9], *etc.* Since the pioneering work of Hofmeister in 1888, the recurring trend of the effectiveness of anions or cations on various physical properties is known as the Hofmeister series [10]. From then on, numerous studies have been carried out to explore the mechanism of this important effect on molecular interactions by various experimental and theoretical methods [11–22]. Considerable thermodynamic or energetic information about this effect is known in spite of its complicated detailed mechanism at the molecular level [23–33].

L-Carnitine (scheme 1) is a typically zwitterionic and osmotically active compound that induces fluid flow across the peritoneum [34,35]. It acts as detoxicant of non-metabolizable acyl Coenzyme A [36]. Via translocation of long-chain fatty acids across the mitochondrial inner membrane, it plays an important role in human metabolism, helping the body turn fat into energy [37]. Its transport in human placental brush-border membranes is mediated by the sodium-dependent organic cation transporter OCTN2 [38,39], and its transport by rat renal brush border membrane vesicles is stimulated by a Na^+ gradient (extravesicular > intravesicular) [40]. The central carbon and the secondary carnitine metabolisms of *Escherichia coli* is affected under salt stress [41]. L-Carnitine regulates human corneal epithelial cell volume under hyperosmotic stress and ameliorate hyperosmotic stress-induced apoptosis [42].

In this work, we determined dilution enthalpies and the 2nd virial enthalpic coefficients (h_2) of L-carnitine in three aqueous alkali halides solutions of different compositions by isothermal titration calorimetry. The h_2 coefficient is considered to be a good measure of the energetic effect in molecular pairwise interaction in solution [43,44]. Based on h_2 coefficients obtained, we focus on the influences of concentrations of aqueous salt solutions and sizes of cations and anions on the competitive balance between hydrophilic and hydrophobic interactions in pairwise self-association of L-carnitine.

2. Experimental

2.1. Materials

L-Carnitine was purchased from SIGMA–ALDRICH, its mass fraction purity is better than 0.98; NaCl, KCl and NaBr were also from

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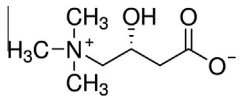

SCHEME 1. Molecular structure of L-carnitine.

SIGMA–ALDRICH, their mass fraction purities are better than 0.995 (table 1). All were used without further purification except for drying over P_2O_5 in a vacuum desiccator for more than 48 h. Aqueous salt (y) solutions ($y + w$) and aqueous L-carnitine (x) solutions ($x + y + w$) were prepared by weight using a Sartorius balance with precision to 0.00001 g. The range of molality (b) of binary aqueous salt solutions is b = (0 to 3.0) mol · kg^{-1}. In all the preparations of solutions, highly purified water (Millipore Elix5/Milli-Q Academic system) was used, with its electrical conductivity $L = 0.056$ μS · cm^{-1} at $T = 298.15$ K.

2.2. Methods

The dilution enthalpies were determined at $T = (298.15 \pm 0.01)$ K and under $p = (0.100 \pm 0.005)$ MPa by an isothermal titration calorimeter (ITC200, MicroCal), which requires only 40 μL of liquid sample. All the solutions were degassed by ultrasound before use. Both the sample (reaction) and the reference cells were loaded with 200 μL of pure water (w), or a binary aqueous salt (y) solution z ($z = y + w$) that serves wholly as "solvent", and the 40 μL syringe was filled with a binary or ternary titrant solution ($x + w$ or z) that was prepared by the same solvent (w or z). A run of titration consisted of consecutive injections of 2 μL of titrant solution ($x + w$ or z) and 5 s duration each, with an interval of 2 min between. The enthalpy effect ($\Delta H(m_{N-1}, m_N)$, J) per injection, which corresponds to the change in molality of the titrated solution in the sample cell from m_{N-1} to m_N, was determined by automatic peak integration of thermal power (P/μcal · s^{-1}) vs time (t/min) curve. The thermal effects from the friction in the process of injection were considered to be negligible. The consecutive dilution enthalpies ($\Delta H(m_{N-1}, m_N)$) of each titrant solution ($x + w$ or z) of the same initial molality (m_0) were measured in parallel for three times, from which the average values of $\Delta H(m_{N-1} \to m_N)$ were calculated. The molality of solution ($x + w$ or z) in the sample cell after the Nth titration can be calculated from the equation,

$$m_N = 10^6 N n_x / \left(10^{-6} V_{\text{sam}} \rho_z + 10^{-6} N V_{\text{inj}} \rho_0 - N n_x M_x\right), \tag{1}$$

in which, N is the number of injections, $N = 1, 2, 3, \ldots 20$; V_{sam} is the volume of sample cell, $V_{\text{sam}} \approx 200$ μL; ρ_z is the density (g · cm^{-3}) of solvent (z) ($z = w$ or $y + w$), and ρ_0 is the density (g · cm^{-3}) of titrant solution ($x + w$ or z) in the syringe, both of which were measured by a precise vibration tube density meter (Anton Paar DMA 5000 M) (see table S2 in Supporting Information); M_x is the molar mass (g · mol^{-1}) of solute x; n_x is the amount of substance (moles) of solute (x) in each injection volume ($V_{\text{inj}} \approx 2$ μL),

$$n_x = 10^{-6} V_{\text{inj}} \rho_0 m_0 / \left(1 + 10^{-6} V_{\text{inj}} \rho_0 m_0 M_x\right), \tag{2}$$

where m_0 is the molality (mol · kg^{-1}) of titrant solution ($x + z$) in the syringe.

Based on the framework of McMillan–Mayer' theory [43], the thermodynamic formula commonly used to deal with the excess enthalpy of a binary solution containing solute x and solvent z can be expressed as follows,

$$H^E(m) = h_2 m + h_3 m^2 + \cdots, \tag{3}$$

where h_2, h_3, *etc.* are known as the 2nd, 3rd and higher order virial enthalpic coefficients respectively, m is the molality of solution ($x + z$). To evaluate these interaction coefficients, molar dilution enthalpies ($\Delta H(m_{N-1} \to m_N)$) of the binary solution ($x + z$) per injection in ITC are necessary [45],

$$\begin{aligned}\Delta H(m_{N-1} \to m_N) &= \Delta H(m_{N-1}, m_N)/n_x = H^E(m_N) - H^E(m_{N-1}) \\ &= (N-1)\left[h_2(m_N - m_{N-1}) + h_3\left(m_N^2 - m_{N-1}^2\right)\right] \\ &\quad + \left[h_2(m_N - m_0) + h_3\left(m_N^2 - m_0^2\right)\right].\end{aligned} \tag{4}$$

In principle, regression analysis can be conducted according to equation (5), from which enthalpic virial coefficients at different levels (h_2, h_3, *etc.*) can be evaluated. Taking into consideration that the volume of solution in the sample cell is much larger than that per injection, *i.e.* $V_{\text{sam}} \gg V_{\text{inj}}$, we have the following equations approximately,

$$m_N = N m_1 \text{ and } m_{N-1} = (N-1) m_1. \tag{5}$$

Therefore, equation (4) can be reduced to

$$\begin{aligned}\Delta H(m_{N-1} \to m_N) &= h_2[(2N-1)m_1 - m_0] \\ &\quad + h_3\left\{[3N(N-1)+1]m_1^2 - m_0^2\right\}.\end{aligned} \tag{6}$$

If $m_1 \ll 1$ mol · kg^{-1}, equation (6) can be simplified further,

$$\Delta H(m_{N-1} \to m_N) = 2h_2 m_1 N - [h_2(m_1 + m_0) + h_3 m_0^2]. \tag{7}$$

According to equation (7), $\Delta H(m_{N-1} \to m_N)$ can be plotted as a linear function of N, from which the values of h_2, h_3 can be obtained.

$$h_2 = \frac{\text{slope}}{2m_1} \quad \text{and} \quad h_3 = \frac{\text{intercept} + \frac{\text{slope}}{2m_1}(m_1 + m_0)}{m_0^2}. \tag{8}$$

In this work, we discuss the h_2 coefficient only since the h_3 coefficient is involved in more complicated interactions of (solute + solute) and (solute + solvent) in solution.

3. Results and discussion

3.1. Experimental dilution enthalpies and calculated virial enthalpic coefficients

All the average values of $\Delta H(m_{N-1} \to m_N)$ obtained from three parallel determinations of ITC and other related data are listed together in table S1 (see Supporting Information). The typical ITC curve of L-carnitine in aqueous NaCl solution (m_0 = 0.7636 mol · kg^{-1}, $b = 0.5000$ mol · kg^{-1}) at $T = (298.15 \pm 0.01)$ K and under $p = (0.100 \pm 0.005)$ MPa, and the fitting plot of successive molar dilution enthalpies ($\Delta H(m_{N-1} \to m_N)$) vs injection

TABLE 1
The sources and purity of experimental materials.

Compounds	Commercial mass fraction purity	Purification methods	Experimental mass fraction purity	Analytic methods
L-Carnitine (inner salt)	⩾0.98	Drying over P_2O_5 under vacuum for more than 48 h	No significant improvement	HPLC
NaCl	⩾0.995	The same with above	0.996	XRD
KCl	⩾0.995	The same with above	0.996	XRD
NaBr	⩾0.995	The same with above	0.996	XRD