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Dilution thermodynamics of the biologically relevant cation mixtures

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ABSTRACT

The ionic composition of intracellular space is rigorously controlled by a variety of processes consuming large quantities of energy. Since the energetic efficiency is an important evolutional criterion, therefore the ion fluxes within the cell should be optimized with respect to the accompanying energy consumption.

In the paper we present the experimental evidence that the dilution enthalpies of the biologically relevant ions; i.e. calcium and magnesium depend on the presence of monovalent cations; i.e. sodium and potassium. The heat flow generated during the dilution of ionic mixtures was measured with the isothermal titration calorimetry. When calcium was diluted together with potassium the dilution enthalpy was drastically reduced as the function of the potassium concentration present in the solution. No such effect was observed when the potassium ions were substituted with sodium ones. When the dilution of magnesium was investigated the dependence of the dilution enthalpy on the accompanying monovalent cation was much weaker. In order to interpret experimental evidences the ionic cluster formation is postulated. The specific organization of such cluster should depend on ions charges, sizes and organization of the hydration layers.

1. Introduction

The intracellular ionic homeostasis provides a stable environment for molecular processes, including proper gene expression, protein functioning, and control of the metabolic pathways via the second messenger systems [1–3]. The importance of the intracellular ionic homeostasis is reflected by the amount of the energy utilized by the cell for its maintenance. For example about thirty percent of energy dissipated by excitable cells is consumed by the $Na^{+}K^{+}$ATPase alone [4–6]. In addition, ions play a number of physiological functions. The electrochemical potential differences of potassium and sodium across the plasma membrane of excitable cells make the action potential possible [7], magnesium is an universal enzymatic cofactor [8], whereas other ions, i.e. calcium, play a crucial role in the control of supra-molecular aggregates topology and stability [1–3,9,10].

In order to maintain the osmotic balance the intracellular ionic strength is maintained constant over time preventing the cell volume change [11]. The maintenance of intracellular ionic homeostasis requires continuous flow of the metabolic energy to compensate for dissipative processes [4,5]. The stability of the intracellular ionic homeostasis is critical for the cell survival, as illustrated by the significant alteration of the intracellular ionic composition accompanying cell progression toward apoptosis [12]. Things are different when it comes to calcium. Calcium concentration fluctuates in space and time throughout the whole cell volume as an important element of the intracellular signal propagation pathways [4,6,13,14]. Its concentration is low in the resting state, normally not exceeding the value of 10 nM. During signal propagation calcium concentration rises by three orders of magnitude reaching transiently the concentrations of 0.1–1 μM [15]. Both concentrations are too small to have any effect on the overall osmotic balance ensuring the homeostasis of all the remaining ions. However, the maintenance of calcium homeostasis itself requires an efficient system of pumps, channels and reservoirs enabling the effective signal propagation [6–8]. Large and frequent fluctuations of calcium concentration within the cell require large quantities of energy so the subsequent calcium dilutions and sequestration from the cytoplasm are possible.

The energetics of ion concentration changes depends on the water structure, water-ion and ion–ion interactions [16–19]. It has been shown elsewhere that the ionic composition of cytoplasm affects variety of processes including such fundamental ones as solubility of macromolecules and assembly of supramolecular aggregates [6,20–22]. In most theoretical and experimental studies it has been assumed that ions are hydrated independently, as in diluted solutions, where the ideal gas approximation can be used [16–19]. Recently, using combined terahertz and femtosecond

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infrared spectroscopic techniques it has been shown that the effect of ions and counter-ions on water organization can be interdependent [23].

In the paper we aim to determine the effect of the ionic composition of the solution on the dilution enthalpy of biologically important ions. The experimental results obtained for the selected mixtures of monovalent and divalent cations upon dilution show that the recorded enthalpy changes are different from the calculated ones, based on the assumption of the enthalpy additivity. The highest deviations from the expected enthalpy values were recorded for the mixtures of K^+/Ca^{2+}. The difference between the dilution enthalpies of monovalent and divalent cations alone and when mixed together may indicate the cation dependent water organization. The dilution enthalpy values recorded for K^+/Ca^{2+} mixtures were altered most significantly which may provide a thermodynamic argument for the specific ionic composition of the intracellular environment.

2. Materials and methods

2.1. Materials

All the chemicals used in this study were of analytical grade and were used as received without any further purification. Sodium chloride (≥99.0%) and calcium chloride (≥97.0%) were purchased from Chempur (Piekary Slaskie, Poland). HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, ≥99.0%), potassium chloride (≥99.5%) and magnesium chloride hexahydrate (≥99.0%) were ordered from POCH (Gliwice, Poland). All sample solutions were prepared with deionized water, the conductivity of which was less than 2 μS/cm.

2.2. Isothermal titration calorimetry (ITC) measurements

The titration measurements were performed using Thermal Activity Monitor TAM III, equipped with isothermal titration unit (TA Instruments, New Castle, USA). The reaction cell was filled with 800 μl of water while the syringe was loaded with the solution of salt or the mixture of salts. The salt solution was titrated into the reaction cell in a series of 15 injections (10 μl per injection), except the reverse titrations, where the water was titrated into the salt solution. Each injection was completed within 10 s under the continuous steering, whereas the time delay between injections was set to 600 s. All experiments were performed at 298.15 K. Each of the titration experiments was preceded by one 1 μl injection, neglected in further data analysis.

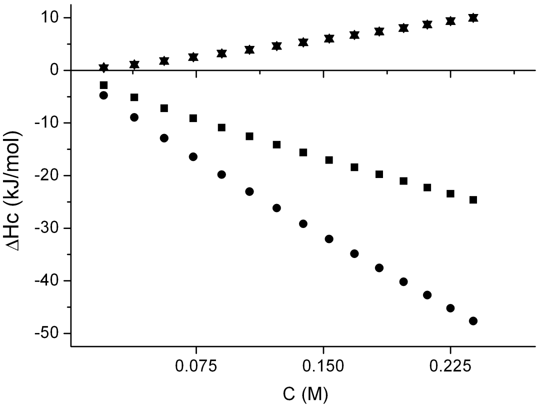

Fig. 1. Cumulative enthalpy changes calculated from the dilution experiments carried out for selected salt solutions as a function of the salt concentration in the reaction cell. NaCl (▲ filled triangle), KCl (▼ reversed filled triangle), $MgCl_2$ (● filled circle), and $CaCl_2$ (■ filled square).

2.3. Data analysis

The heat released/consumed in the course of experiment was calculated as the area under the thermogram peaks. Positive heat flow values on the thermograms represent the exothermic response, whereas the negative values stand for the endothermic ones. Enthalpies of dilution ($\Delta_{dil}H_{exp}$) were calculated from the titration experiments, where 1.5 M salt solutions were diluted in 15 consecutive steps. Given enthalpies are the values obtained in the first titration step, with the assumption, that the salt densities are equal to 1 g/ml. The cumulative enthalpy was calculated as the sum of consecutive enthalpy changes during the course of titration (e.g. Fig. 1). To fit the resulting data, namely the dependence of the cumulative enthalpy changes on the ion concentration in the titration chamber, the linear regression was performed. As the result, the slopes of the corresponding linear functions were calculated for different salts mixtures and compared (e.g. Fig. 2). Measurement uncertainties were estimated in separate series of experiments, where the dilution of the 1.5 M potassium chloride solution was performed in triplicate. The calculated measurement error was less than 5%. This error value was assumed for all other titration experiments performed.

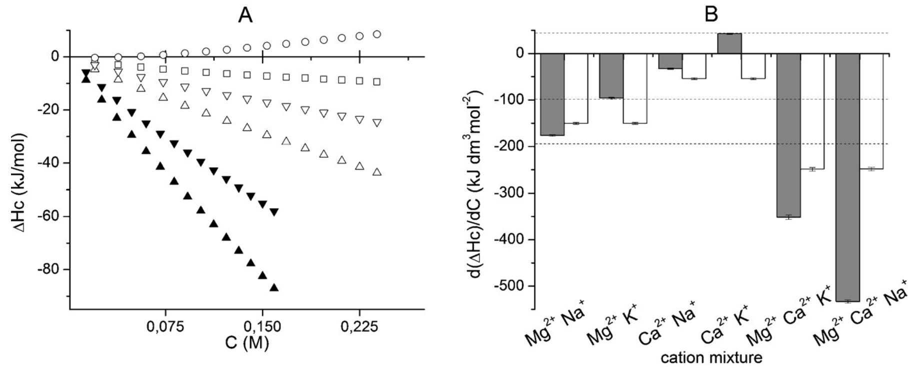

Fig. 2. Cumulative enthalpy changes and their rates calculated from the dilution experiments carried out for the selected mixtures of salt solutions. (A) Cumulative enthalpy changes plotted as a function of the divalent ion concentration in the reaction cell. $CaCl_2/NaCl$ (□ square), $CaCl_2/KCl$ (○ circle), $MgCl_2/NaCl$ (△ triangle), $MgCl_2/KCl$ (▽ reversed triangle), $CaCl_2/MgCl_2/NaCl$ (▲ filled triangle), $CaCl_2/MgCl_2/KCl$ (▼ reversed filled triangle). (B) Rate of the cumulative enthalpy change due to the salt concentration increase in the reaction cell upon titration (slope of a plot presented in Fig. 2A). The filed bars represent experimentally determined values, whereas the empty bars represent the calculated ones, with the assumption that the two indicated salts in a mixture dissolve independently. The uppermost, the middle and the lowest horizontal lines represent experimentally determined slope values, where $MgCl_2$, $CaCl_2$, and KCl were diluted independently.