Biophysical Chemistry 180–181 (2013) 102–109

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Low concentrated hydroxyectoine solutions in presence of DPPC lipid bilayers: A computer simulation study

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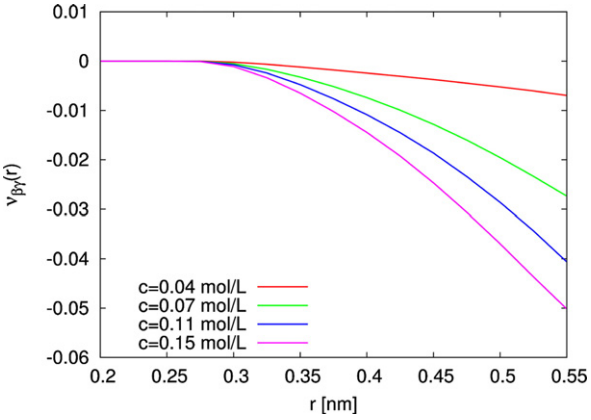
HIGHLIGHTS

- Numerical study of the kosmotropic properties of hydroxyectoine in presence of DPPC lipid bilayers
- Strengthening of the water hydrogen bond network in presence of hydroxyectoine
- Validation of preferential exclusion mechanism for hydroxyectoine in terms of Kirkwood–Buff theory
- Increased surface pressure for DPPC lipid bilayers with increased hydroxyectoine concentration
- Visibility of co-solute effects on DPPC lipid bilayers for low and physiological concentrations

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 June 2013
Received in revised form 5 July 2013
Accepted 5 July 2013
Available online 13 July 2013

Keywords:
Compatible solutes
Osmolytes
Molecular Dynamics simulations
DPPC lipid bilayers
Kosmotropes

ABSTRACT

The influence of hydroxyectoine on the properties of the aqueous solution in presence of DPPC lipid bilayers is studied via semi-isotropic constant pressure (NPT) Molecular Dynamics simulations. We investigate the solvent–co-solute behavior in terms of Kirkwood–Buff integrals as well as hydrogen bond life times for an increasing hydroxyectoine concentration up to 0.15 mol/L. The observed preferential exclusion mechanism identifies hydroxyectoine as a kosmotropic osmolyte. Our findings with regard to the DPPC lipid bilayer indicate an increase of the surface pressure as well as the solvent accessible surface area in presence of higher hydroxyectoine concentrations. The results are in agreement to the outcome of recent experiments. With this study, we are able to validate the visibility of co-solute–solute–solvent effects for low and physiologically relevant osmolyte concentrations.

1. Introduction

Osmolytes allow extremophilic microorganisms to resist harsh living conditions [1,2]. Typical examples for these species are ectoine and hydroxyectoine which are zwitterionic, strong water binding and low-molecular weight organic molecules. The functionalities of these

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http://dx.doi.org/10.1016/j.bpc.2013.07.001

co-solutes in living organisms among others are given by the protection of protein conformations [1–5] and the fluidization of lipid membranes [6,7]. The protective properties become mainly important under environmental stress conditions, e. g. high temperature, extreme dryness and salinity [8]. Various studies have validated that combinations of osmolytes can be found in biological cells. The concentration of a single osmolyte in these mixtures varies between 0.1 and 1.0 mol/L ([9] and references therein). Due to the fact that several osmolytes are not affecting the cell metabolism, specific molecules like the ectoines are also commonly called compatible solutes.

Recent studies were focusing on the molecular functionality of the ectoines as well as the analysis of the protective behavior [3,4]. The theoretical framework for the explanation of co-solute–solute effects has been mainly established in terms of the preferential exclusion [10] and the transfer free energy model [11].

These models focus on the strong ordering of the local water shell around the osmolytes and the exclusion from the immediate hydration shell of the solute which results in a preferential hydration behavior and a stabilization of the solutes native structure [10,11]. Among these, more refined versions of these theories have been additionally published which explicitly rely on the properties of chaotropic and kosmotropic co-solute behavior and the corresponding interaction with macromolecular surfaces [12–15]. Co-solutes which strengthen the water hydrogen network are called kosmotropes (structure makers) while osmolytes which weaken the water network structure are called chaotropes (structure breaker). The separation of co-solutes into these two species is not unique and straightforward [15]. Interactions and binding properties between kosmotropes and chaotropes can be predicted by the 'law of matching water affinities' [13,15]. A main point of this law is the investigation of the corresponding hydration free energies which loosely depend among other factors on the molecular charge [15]. One of the major achievements of the 'law of matching water affinities' is the molecular description of a repulsive behavior for kosmotropic osmolytes like ectoine from polar surfaces and vice versa, the attraction of chaotropic agents like urea. The preferential binding of urea has been validated in recent computer simulations [16] while additional studies have observed a kosmotropic behavior for ectoine in terms of a preferential exclusion mechanism around Chymotrypsin Inhibitor II [4].

It has been stated that kosmotropic co-solutes typically accumulate in the second or third hydration shell of the solvated macromolecule [13]. With regard to their high charging and affinity for water molecules, it is assumed that this appearance strongly influences the first and the second hydration shell of the polar solute. The consequence of this behavior is given by a diminished number of solute–water hydrogen bonds which is compensated by a significant shrinkage of the solute surface to maintain a constant hydrogen bond surface density. It is commonly believed that this shrinkage in size is the molecular reason for the preservation of native protein conformations in presence of kosmotropic co-solutes [13,15].

Although the stabilizing effects on proteins in presence of specific osmolytes have been studied extensively before, less is known about compatible solutes and their interactions with lipid bilayers. A small number of theoretical studies have focused on sugars like trehalose and their interactions with lipid membrane bilayers and monolayers [17–23]. For high molar concentrations of trehalose, it has been found that replacement of water molecules by the formation of additional sugar-membrane hydrogen bonds plays a major role [18]. Despite this interpretation, it has been also discussed that the effects observed in sugar-DPPC mixtures can be only systematically explained by an interplay of several mechanisms [23].

In addition to theoretical studies, experimental findings have indicated a significant broadening of the liquid expanded (LE)–liquid condensed (LC) phase transition of monolayers in presence of ectoine and hydroxyectoine [6,7]. This was mainly indicated by the study of the corresponding surface pressure area isotherms. A main result of these studies was the observation of a surface pressure increase for higher hydroxyectoine concentrations. In addition it was supposed that the domain sizes of the liquid condensed regions significantly shrink in presence of hydroxyectoine which corresponds to a variation of the line tension [6,24–26]. With regard to the biological function, the above mentioned effects are of particular importance for signaling processes and cell repair [2,6].

With regard to the preferential exclusion/binding behavior of osmolyte–solute–solvent mixtures, computer simulations allow a detailed study of the corresponding molecular mechanisms. A theoretical framework which allows to distinguish between exclusion and binding behavior has been established in terms of the Kirkwood–Buff theory of solutions [27–29]. The corresponding analysis has been successfully applied to the study of urea and polyglycine interactions [16]. It has been shown that the calculation of the Kirkwood–Buff integrals allows the effective determination of transfer free energies in addition to the detection of kosmotropic as well as chaotropic behavior [4,16].

In this paper, we study the properties of an aqueous hydroxyectoine solution in presence of DPPC lipid bilayers via semi-isotropic constant pressure (NPT) all-atom Molecular Dynamics simulations. The concentration of hydroxyectoine is low but physiologically relevant [9] with a maximum value of 0.15 mol/L. We have been inspired to use these small concentrations due to recent experimental findings for aqueous hydroxyectoine–DPPC monolayer mixtures [6,7]. Most of the simulation studies usually employ high co-solute concentrations which are often above one mole per liter to study pronounced behavior at unphysiological conditions [3,4,16]. With this study, we are able to validate the observation of effects at smaller concentrations in agreement to experimental findings.

Our main result is given by the characterization of hydroxyectoine as a kosmotropic osmolyte which strengthens the water hydrogen bond network. We are further able to validate a weakening of DPPC–water hydrogen bond interactions in presence of hydroxyectoine. With regard to the DPPC lipid bilayer properties, our results validate an increase of the surface pressure and the solvent-accessible surface area in agreement to the experimental findings [6,7,5]. We emphasize the importance of electrostatic interactions between hydroxyectoine and DPPC molecules for the understanding of the observed effects by the calculation of the lipid bilayer electrostatic potential.

The paper is organized as follows. In the next section, we shortly introduce the theoretical background. In the third section we illustrate the simulation details and the methodology. The results for the solvent properties and the DPPC lipid bilayer are presented and discussed in the fourth section. We briefly conclude and summarize in the last section.

2. Theoretical Background

2.1. Kirkwood–Buff integrals and preferential binding parameter

The evaluation of statistical mechanics methods on the co-solvent and solvent distribution function allows important insights into the preferential exclusion as well as binding behavior in terms of the corresponding Kirkwood–Buff theory which has been introduced in the early 1950s [27,28]. The radial distribution function of molecules or atoms β around solutes α can be expressed by

$$g_{\alpha\beta}(r) = \frac{\rho_\beta(r)}{\rho_{\beta,\infty}} \tag{1}$$

where $\rho_\beta(r)$ denotes the local density of β at a distance r around the solute and $\rho_{\beta,\infty}$ the global density in the bulk phase [30]. The Kirkwood–Buff integral is given by the integration of Eq. (1)

$$G_{\alpha\beta} = \lim_{R\to\infty} G_{\alpha\beta}(R) = \lim_{R\to\infty} \int_{r=0}^{r=R} 4\pi r^2 \left(g_{\alpha\beta}(r) - 1\right)\, dr \tag{2}$$