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ORIGINAL PAPER

A combined experimental and theoretical study on the complexation of Li^+ with valinomycin

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Abstract From extraction experiments in the two-phase water–nitrobenzene system and γ-activity measurements, the stability constant of the valinomycin–lithium complex in nitrobenzene saturated with water was determined. Further, the structure of the resulting complex was indicated by means of the density functional level of theory (DFT) calculations.

Keywords Antibiotics · Macrocycles · Stability constant · Ab initio calculations · Complex structure

Introduction

Structures and substances mediating ion transfer across a biological membrane still attract scientific interest. Among them, valinomycin (abbrev. **1**, see Scheme 1) has been one of the first to be recognized as an ion carrier or ionophore. The ability of valinomycin to carry ions across a membrane is primarily due to its forming of a molecular complex with them, secondarily to the lipophilic nature of the outer rim of its depsipeptide ring, which secures its embedding into the membrane. It had been originally believed that a complex is formed selectively with potassium cation, but further studies have shown that other metal ions bind to valinomycin as well [1–4].

Recently, experimental evidences for a valinomycin–proton complex as well as for some unusual divalent cation complexes of valinomycin have been reported [5, 6]. However, up to now, the structure of the valinomycin–lithium complex has not been solved. In the present work, the stability constant of the $\mathbf{1}\cdot Li^+$ complex species is determined in the organic phase of the water–nitrobenzene extraction system. Moreover, applying quantum mechanical density functional level of theory (DFT) calculations, the most probable structure of the above-mentioned cationic complex species is derived.

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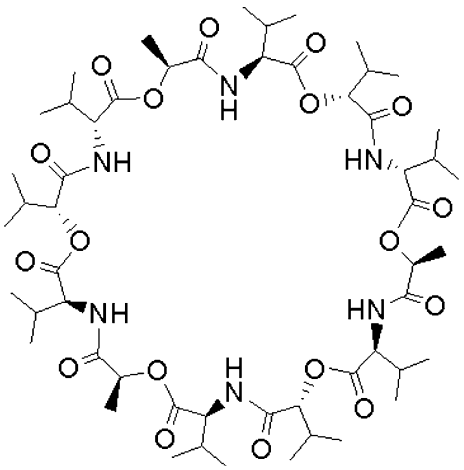
Results and discussion

Extraction experiments

In terms of previous results [5, 7, 8], the two-phase water–LiCl/nitrobenzene–NaDCC–**1** (valinomycin) extraction system, chosen for the determination of the stability constant of the complex $\mathbf{1}\cdot Li^+$ in nitrobenzene saturated with water, can be characterized by the main chemical equilibrium (1) to which the equilibrium extraction constant (Eq. 2) corresponds; aq and nb denote the presence of the species in the aqueous and nitrobenzene phases, respectively:

$$Li^+(aq) + \mathbf{1}\cdot Na^+(nb) \rightleftarrows \mathbf{1}\cdot Li^+(nb) + Na^+(aq); \quad K_{ex}(Li^+,\ \mathbf{1}\cdot Na^+) \tag{1}$$

$$K_{ex}(Li^+,\ \mathbf{1}\cdot Na^+) = \frac{[\mathbf{1}\cdot Li^+]_{nb}[Na^+]_{aq}}{[Li^+]_{aq}[\mathbf{1}\cdot Na^+]_{nb}} \tag{2}$$



Scheme 1

It is necessary to emphasize that **1** is a considerably hydrophobic ligand, practically present in the nitrobenzene phase only, where this ligand forms—with Li^+ and Na^+—the relatively stable complexes $\mathbf{1}\cdot Li^+$ and $\mathbf{1}\cdot Na^+$.

Taking into account the conditions of electroneutrality in the organic and aqueous phases of the system under study, the mass balances of Li^+ and Na^+ ions at equal volumes of the nitrobenzene and aqueous phases, as well as the measured equilibrium distribution ratio of sodium, $D_{Na} = [\mathbf{1}\cdot Na^+]_{nb}/[Na^+]_{aq}$, combined with Eq. 2, we get the final expression for the above-mentioned extraction constant (Eq. 3); $C^{in,\,aq}_{LiCl}$ is the initial concentration of LiCl in the aqueous phase and $C^{in,\,nb}_{NaDCC}$ denotes the initial concentration of NaDCC in the organic phase of the system under consideration:

$$K_{ex}(Li^+,\ \mathbf{1}\cdot Na^+) = \frac{1}{D_{Na}}\frac{C^{in,\,nb}_{NaDCC}}{(1+D_{Na})C^{in,\,aq}_{LiCl} - C^{in,\,nb}_{NaDCC}} \qquad (3)$$

From the extraction experiments and γ-activity measurements by using Eq. 3, the following value of the constant $K_{ex}(Li^+,\ \mathbf{1}\cdot Na^+)$ was evaluated as $\log K_{ex}(Li^+,\ \mathbf{1}\cdot Na^+) = -1.1 \pm 0.1$. Furthermore, with respect to [5, 7, 8], for the exchange extraction constant $K_{ex}(Li^+,\ Na^+)$ corresponding to the equilibrium $Li^+(aq) + Na^+(nb) \rightleftarrows Li^+(nb) + Na^+(aq)$ and for the extraction constant $K_{ex}(Li^+,\ \mathbf{1}\cdot Na^+)$ defined above, as well as for the stability constants of the complexes $\mathbf{1}\cdot Na^+$ and $\mathbf{1}\cdot Li^+$ in nitrobenzene saturated with water, denoted by $\beta_{nb}(\mathbf{1}\cdot Na^+)$ and $\beta_{nb}(\mathbf{1}\cdot Li^+)$, one obtains Eq. 4:

$$\begin{aligned}\log \beta_{nb}(\mathbf{1}\cdot Li^+) = {} & \log \beta_{nb}(\mathbf{1}\cdot Na^+) \\ & + \log K_{ex}(Li^+,\ \mathbf{1}\cdot Na^+) \\ & - \log K_{ex}(Li^+,\ Na^+)\end{aligned} \qquad (4)$$

Using the value $\log K_{ex}(Li^+,\ Na^+) = -0.7$ inferred from [7], the constant $\log K_{ex}(Li^+,\ \mathbf{1}\cdot Na^+)$ given above and $\log \beta_{nb}(\mathbf{1}\cdot Na^+) = 6.7$ [9], determined from the distribution of sodium picrate in the water–nitrobenzene extraction system also containing **1**, and applying Eq. 4, we obtain the stability constant of the $\mathbf{1}\cdot Li^+$ complex in water-saturated nitrobenzene at 25 °C as $\log \beta_{nb}(\mathbf{1}\cdot Li^+) = 6.3 \pm 0.1$. This means that in the above-mentioned medium, the stability constant of the considered complex $\mathbf{1}\cdot Li^+$ is somewhat lower than that of the $\mathbf{1}\cdot Na^+$ complex.

Quantum mechanical calculations

The quantum mechanical calculations were carried out at the density functional level of theory (DFT, *B3LYP* functional) using the Gaussian 03 suite of programs [10]. The 6–31G(d) basis set was used and the optimizations were unconstrained. Although the possible influence of a polar solvent on the detailed structures of **1** and the Li^+ complex species of **1** could be imagined, our quantum mechanical calculations in similar cases, performed in an analogous way, showed very good agreement of the experiment with the theory [11, 12].

Recently, the hydration number of the valinomycin–lithium complex in the organic phase of the water–nitrobenzene extraction system was evaluated as $h(\mathbf{1}\cdot Li^+) = 3.1 \pm 0.1$ (P. Selucký, 2007, private communication) by means of the method published elsewhere [13]. Thus, in this study, let us consider further both the "nonhydrated" state ($\mathbf{1}\cdot Li^+$) and the "hydrated" state ($\mathbf{1}\cdot Li^+\cdot 3H_2O$) of the mentioned complex species.

In the model calculations, we optimized the molecular geometry of the parent valinomycin ligand **1** and its complex with Li^+. The optimized structure of **1** is shown in Fig. 1 together with the lengths of the respective hydrogen bonds. From this figure, it follows that the predicted structure of free **1** having C_3 symmetry is very much like that ingeniously derived by the early researchers from their experimental data [3, 4, 14–22]: the molecule adopts a bracelet-like form fortified by six internal hydrogen bonds NH···O (1.98 Å) and three internal hydrogen bonds CH···O (2.52 Å). The ester carbonyl groups are oriented in approximately "radial directions," forming a natural cavity prepared for the coordination of a positive ion.

In Fig. 2, the structure obtained by the full optimization of the $\mathbf{1}\cdot Li^+$ complex is depicted together with the lengths of the corresponding Li^+···O bonds (in Å). Compared to free ligand **1**, the valinomycin part of the complex $\mathbf{1}\cdot Li^+$ is significantly distorted due to the strong Li^+···O bond interactions between the "central" Li^+ cation and four ester carbonyl oxygen atoms of **1**, so that the position of the considered Li^+ ion in the valinomycin cage is extremely eccentric as well. The calculated binding energy of the complex $\mathbf{1}\cdot Li^+$ is -503.7 kJ mol^{-1}.

Finally, the optimized structure of the $\mathbf{1}\cdot Li^+\cdot 3H_2O$ complex species is shown in Fig. 3. In this complex, the

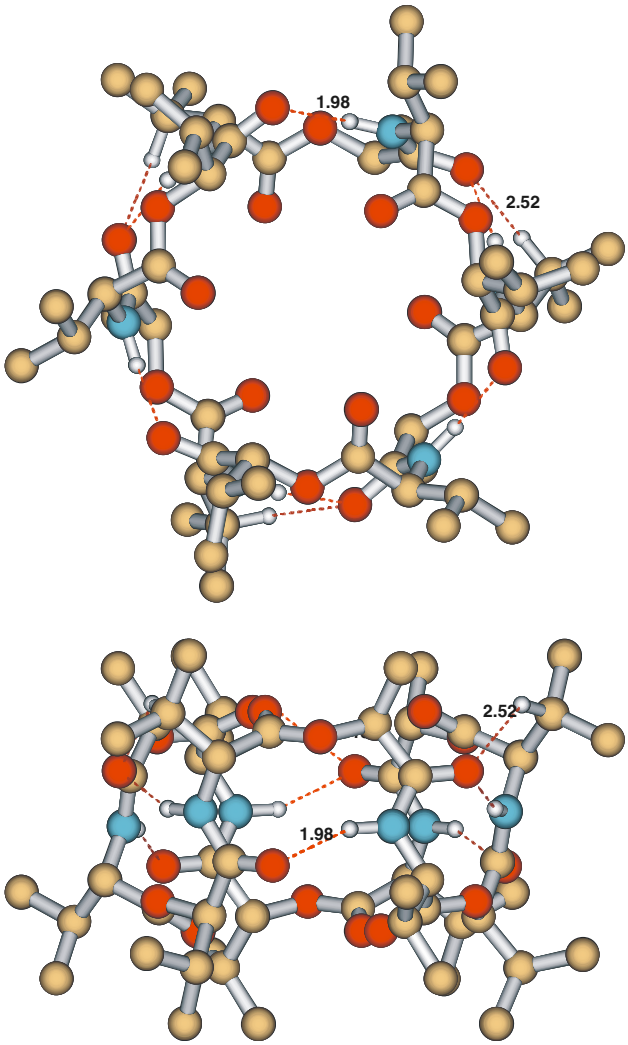


Fig. 1 Two projections of the density functional level of theory (DFT)-optimized structure of free **1** (*B3LYP*/6–31G(d)) (hydrogen atoms omitted for clarity except nine hydrogens taking place in six internal hydrogen bonds NH···O (1.98 Å) and three internal hydrogen bonds CH···O (2.52 Å))

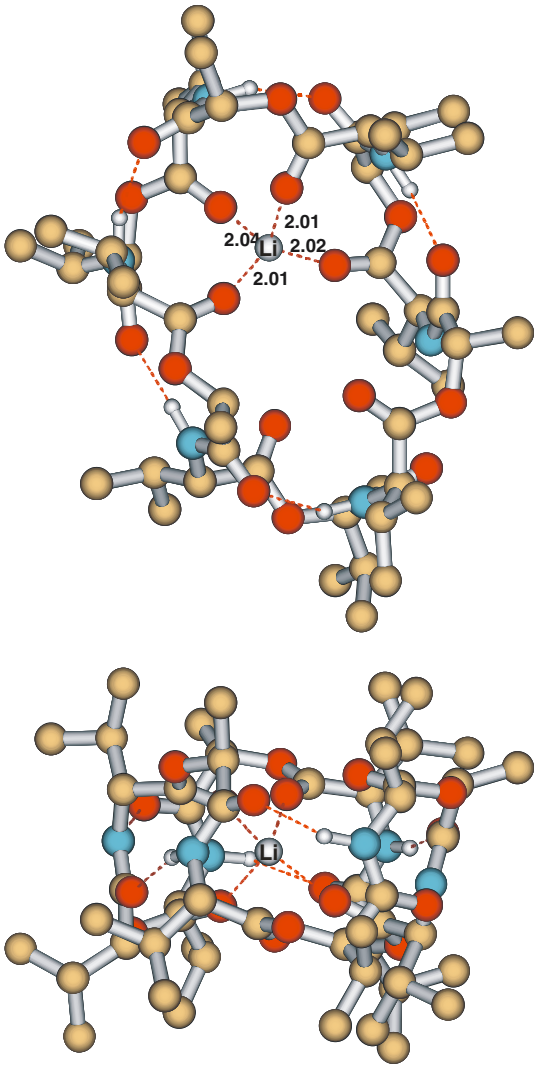


Fig. 2 Two projections of the DFT-optimized structure of the **1**·Li^+ complex (*B3LYP*/6–31G(d)) (hydrogen atoms omitted for clarity except hydrogens taking place in internal hydrogen bonds NH···O and CH···O)

"central" Li^+ ion is bound by strong bonds to three oxygen atoms of the respective water molecules (1.93, 1.97, and 1.94 Å) and to one ester carbonyl oxygen (1.97 Å) of the parent valinomycin ligand **1**. Besides this, the **1**·Li^+·$3H_2O$ cationic complex system is evidently stabilized by six strong hydrogen bonds OH···O, as also illustrated in detail in Fig. 3. From this point of view, it is necessary to emphasize that the optimized structure of the **1**·Li^+·$3H_2O$ cationic complex species stabilized by the mentioned hydrogen bonds is apparently much more real than that of the complex **1**·Li^+. This fact confirms the calculated binding energy of the **1**·Li^+·$3H_2O$ species (−741.5 kJ mol^{-1}), which is substantially higher than the binding energy given above corresponding to the **1**·Li^+ complex.

Experimental

Cesium dicarbollylcobaltate (CsDCC) was purchased from Katchem, Řež, Czech Republic. A nitrobenzene solution of HDCC [23] was prepared from CsDCC by the procedure described in [13]. The equilibration of the nitrobenzene solution of HDCC with stoichiometric NaOH, which was dissolved in an aqueous solution of NaCl (0.2 M), yielded the corresponding NaDCC solution in nitrobenzene. Valinomycin (**1**) was supplied by Fluka, Buchs, Switzerland. The other chemicals used (Lachema, Brno, Czech Republic) were of reagent grade purity. The radionuclide $^{22}Na^+$ (DuPont, Belgium) was of standard radiochemical purity.

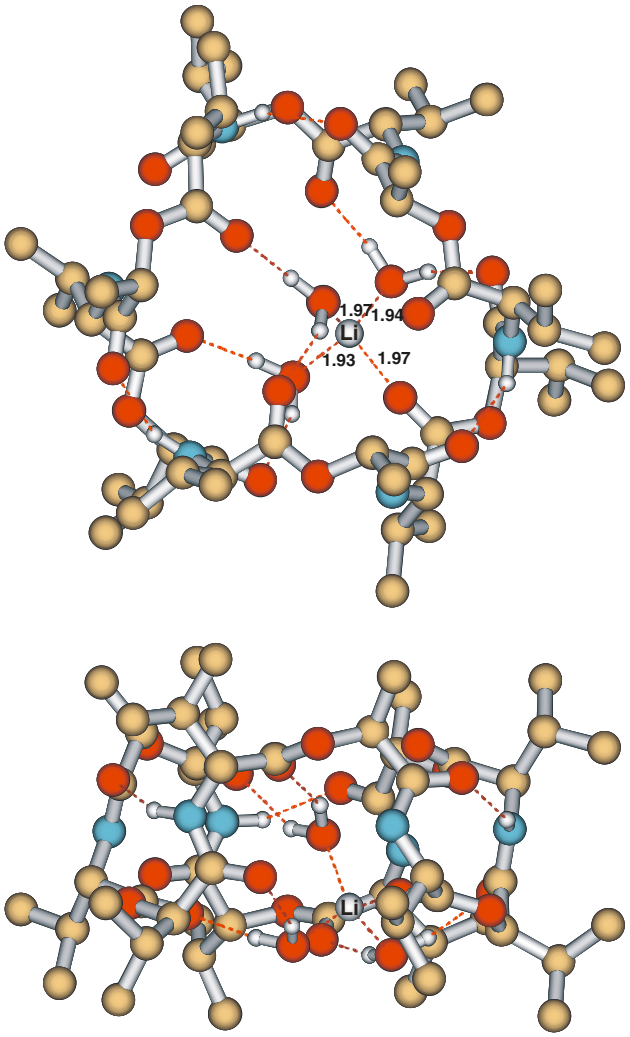


Fig. 3 Two projections of the DFT-optimized structure of the **1**·Li^+·$3H_2O$ complex (*B3LYP*/6–31G(d)) (hydrogen atoms omitted for clarity except hydrogens of three water molecules and hydrogens taking place in internal hydrogen bonds NH···O and CH···O)

The extraction experiments were carried out in 10-cm^3 glass test tubes covered with polyethylene stoppers: 2 cm^3 of an aqueous solution of LiCl of a concentration in the range from 1×10^{-3} to 1×10^{-2} M and microamounts of $^{22}Na^+$ were added to 2 cm^3 of a nitrobenzene solution of **1** and NaDCC, whose initial concentrations also varied from 1×10^{-3} to 1×10^{-2} M (in all experiments, the initial concentration of **1** in nitrobenzene, $C_1^{in,nb}$, was equal to the initial concentration of NaDCC in this medium, $C_{NaDCC}^{in,nb}$). The test tubes filled with the solutions were shaken for 2 h at 25 ± 1 °C using a laboratory shaker. Then, the phases were separated by centrifugation. Afterwards, 1-cm^3 samples were taken from each phase and their γ-activities were measured using a well-type NaI(Tl) scintillation detector connected to a γ-analyzer NK 350 (Gamma, Budapest, Hungary). The equilibrium distribution ratio of sodium, D_{Na}, was determined as the ratio of the measured radioactivities of $^{22}Na^+$ in the nitrobenzene and aqueous samples.

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