

Extraction and DFT study on the complexation of Mg²⁺ 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–magnesium 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 calculations.

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

Introduction

Valinomycin (**1**, Scheme 1) is a dodecadepsipeptide made up of three identical tetradepsipeptides, each of which consists of four sub-units of alternating amino acid and hydroxy acid residues, linked by peptide and ester bonds. The valinomycin molecule thus takes the form of a

36-membered ring and has a multiplicity of conformational possibilities. The ability of valinomycin to carry ions across a membrane is primarily due to its forming of molecular complexes with them and, secondarily, to the lipophilic nature of the outer rim of its depsipeptide ring, which secures its embedding into the membrane. It was originally believed that a complex was formed selectively with potassium cation, but further studies have shown that other metal ions also bind to valinomycin [1–4].

Experimental evidences for a valinomycin–proton complex and for unusual divalent cation complexes of valinomycin have recently been reported [5, 6]. However, up to now, the structure of the valinomycin–magnesium complex has not been elucidated. In this work, the stability constant of the **1**·Mg²⁺ complex in the organic phase of the water–nitrobenzene extraction system was evaluated. Moreover, by applying quantum mechanical density functional level of theory (DFT) calculations, the most probable structure of the above-mentioned cationic complex species was predicted.

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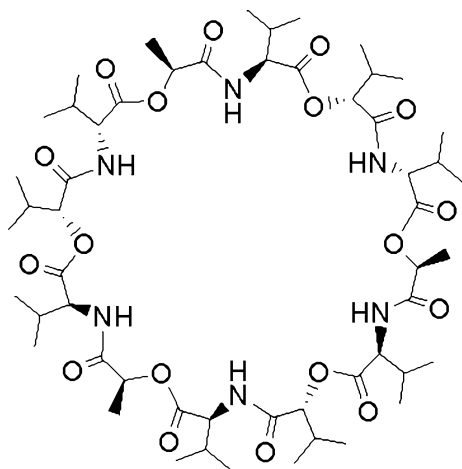
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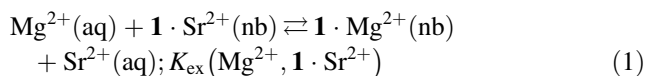
Results and discussion

Extraction experiments

Results from previous papers [7–11] indicate that the two-phase water–Mg(NO₃)₂/nitrobenzene–Sr(DCC)₂–**1** (valinomycin) extraction system, chosen for determination of the stability constant of the complex **1**·Mg²⁺ in nitrobenzene saturated with water, can be characterized by the main chemical equilibrium given by Eq. (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.



Scheme 1



$$K_{\text{ex}}(\text{Mg}^{2+}, \mathbf{1} \cdot \text{Sr}^{2+}) = \frac{[\mathbf{1} \cdot \text{Mg}^{2+}]_{\text{nb}} [\text{Sr}^{2+}]_{\text{aq}}}{[\text{Mg}^{2+}]_{\text{aq}} [\mathbf{1} \cdot \text{Sr}^{2+}]_{\text{nb}}} \quad (2)$$

It is necessary to emphasize that **1** is an extremely hydrophobic ligand, practically present in the nitrobenzene phase only, where this ligand forms—with Mg^{2+} and Sr^{2+} —the relatively stable complexes $\mathbf{1} \cdot \text{Mg}^{2+}$ and $\mathbf{1} \cdot \text{Sr}^{2+}$.

Taking into account the conditions of electroneutrality in the organic and aqueous phases of the system under study, the mass balances of Mg^{2+} and Sr^{2+} ions for equal volumes of the nitrobenzene and aqueous phases, and the measured equilibrium distribution ratio of strontium, $D_{\text{Sr}} = [\mathbf{1} \cdot \text{Sr}^{2+}]_{\text{nb}} / [\text{Sr}^{2+}]_{\text{aq}}$, combined with Eq.(2), we obtain the final expression for the above-mentioned extraction constant (Eq. (3)); $C_{\text{Mg}(\text{NO}_3)_2}^{\text{in, aq}}$ is the initial concentration of $\text{Mg}(\text{NO}_3)_2$ in the aqueous phase and $C_{\text{Sr}(\text{DCC})_2}^{\text{in, nb}}$ denotes the initial concentration of $\text{Sr}(\text{DCC})_2$ in the organic phase of the system under consideration.

$$K_{\text{ex}}(\text{Mg}^{2+}, \mathbf{1} \cdot \text{Sr}^{2+}) = \frac{1}{D_{\text{Sr}} (1 + D_{\text{Sr}})} \frac{C_{\text{Sr}(\text{DCC})_2}^{\text{in, nb}}}{C_{\text{Mg}(\text{NO}_3)_2}^{\text{in, aq}} - C_{\text{Sr}(\text{DCC})_2}^{\text{in, nb}}} \quad (3)$$

From the extraction experiments and γ -activity measurements, and by using Eq. (3), the value of the constant $K_{\text{ex}}(\text{Mg}^{2+}, \mathbf{1} \cdot \text{Sr}^{2+})$ was evaluated as $\log K_{\text{ex}}(\text{Mg}^{2+}, \mathbf{1} \cdot \text{Sr}^{2+}) = -0.4 \pm 0.1$. Furthermore, in accordance with other results [5, 6, 8], for the exchange extraction constant $K_{\text{ex}}(\text{Mg}^{2+}, \text{Sr}^{2+})$ corresponding to the equilibrium $\text{Mg}^{2+}(\text{aq}) + \text{Sr}^{2+}(\text{nb}) \rightleftharpoons \text{Mg}^{2+}(\text{nb}) + \text{Sr}^{2+}(\text{aq})$ and for the extraction constant $K_{\text{ex}}(\text{Mg}^{2+}, \mathbf{1} \cdot \text{Sr}^{2+})$ defined above, and for the stability constants of the complexes $\mathbf{1} \cdot \text{Sr}^{2+}$ and

$\mathbf{1} \cdot \text{Mg}^{2+}$ in nitrobenzene saturated with water, denoted by $\beta_{\text{nb}}(\mathbf{1} \cdot \text{Sr}^{2+})$ and $\beta_{\text{nb}}(\mathbf{1} \cdot \text{Mg}^{2+})$, one obtains Eq. (4).

$$\log \beta_{\text{nb}}(\mathbf{1} \cdot \text{Mg}^{2+}) = \log \beta_{\text{nb}}(\mathbf{1} \cdot \text{Sr}^{2+}) + \log K_{\text{ex}}(\text{Mg}^{2+}, \mathbf{1} \cdot \text{Sr}^{2+}) - \log K_{\text{ex}}(\text{Mg}^{2+}, \text{Sr}^{2+}) \quad (4)$$

Using the value $\log K_{\text{ex}}(\text{Mg}^{2+}, \text{Sr}^{2+}) = -0.6$ inferred from Refs. [10, 11], the constant $\log K_{\text{ex}}(\text{Mg}^{2+}, \mathbf{1} \cdot \text{Sr}^{2+})$ given above and $\log \beta_{\text{nb}}(\mathbf{1} \cdot \text{Sr}^{2+}) = 5.4$ [12], and applying Eq. (4), we obtain the stability constant of the $\mathbf{1} \cdot \text{Mg}^{2+}$ complex species in water-saturated nitrobenzene at 25 °C as $\log \beta_{\text{nb}}(\mathbf{1} \cdot \text{Mg}^{2+}) = 5.6 \pm 0.1$. It means that in this medium the stability constants of the complexes $\mathbf{1} \cdot \text{Mg}^{2+}$ and $\mathbf{1} \cdot \text{Sr}^{2+}$ are comparable.

Quantum mechanical calculations

The quantum mechanical calculations were performed at the density functional level of theory (DFT, B3LYP functional) using the Gaussian 03 suite of software [13]. The 6-31G(d) basis set was used and the optimizations were unconstrained. Although a possible effect of the polar solvent on the detailed structures of **1** and the Mg^{2+} complex of **1** could be imagined, our quantum mechanical calculations in similar cases, carried out in an analogous way, showed very good agreement of experiment with theory [14–16].

Recently, the hydration number of the valinomycin–magnesium complex in the organic phase of the water–nitrobenzene extraction system was determined as $h(\mathbf{1} \cdot \text{Mg}^{2+}) = 2.9 \pm 0.1$ (P. Selucký, 2007, private communication) by means of the method published elsewhere [17]. Hence, in this study, let us consider further both the “nonhydrated” state ($\mathbf{1} \cdot \text{Mg}^{2+}$) and the “hydrated” state ($\mathbf{1} \cdot \text{Mg}^{2+} \cdot 3\text{H}_2\text{O}$) of this magnesium complex.

In the model calculations, we optimized the molecular geometry of the parent valinomycin ligand **1** and its complex with Mg^{2+} . The optimized structure of free **1** having C_3 symmetry, very much like that ingeniously derived by early researchers from their experimental data [18–26], has been presented in our previous paper [27].

In Figs. 1 and 2, two structures (“asymmetrical” and “symmetrical”) obtained by full optimizations of the $\mathbf{1} \cdot \text{Mg}^{2+}$ complex are depicted, together with the lengths of the respective $\text{Mg}^{2+} \cdots \text{O}$ bonds (in Å). In this context it is necessary to emphasize that the binding energies corresponding to the “asymmetrical” and “symmetrical” structures of this $\mathbf{1} \cdot \text{Mg}^{2+}$ complex are $-1,442.6$ and $-1,382.3$ kJ mol^{-1} , respectively. This means that the “asymmetrical” structure of the $\mathbf{1} \cdot \text{Mg}^{2+}$ species shown in Fig. 1 is somewhat energetically favoured (by 62.3 kJ mol^{-1}) in comparison with the “symmetrical” structure illustrated in Fig. 2.

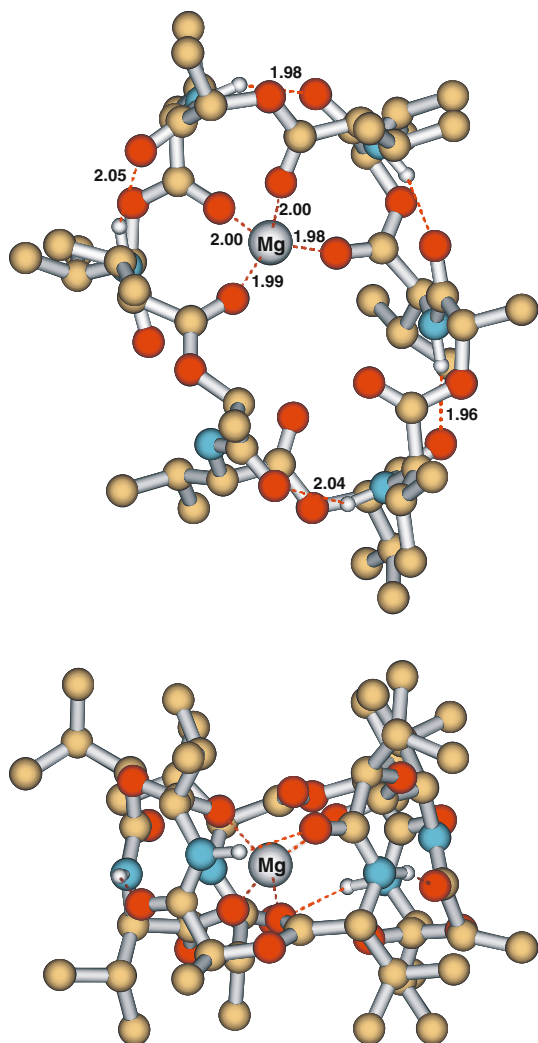


Fig. 1 Two projections of the DFT optimized “asymmetrical” structure of the $1 \cdot \text{Mg}^{2+}$ complex [B3LYP/6-31G(d)] (hydrogen atoms are omitted for clarity except for those taking part in internal hydrogen bonds $\text{NH} \cdots \text{O}$)

Finally, the optimized structure of the $1 \cdot \text{Mg}^{2+} \cdot 3\text{H}_2\text{O}$ complex species is presented in Fig. 3. In this complex, the “central” Mg^{2+} ion is bound by three strong bonds to three oxygen atoms of the respective water molecules (2.00, 2.02, and 2.05 Å) and to two oxygen atoms of the corresponding C=O groups (2.10 and 2.08 Å) of the parent valinomycin ligand **1**. Besides this, the $1 \cdot \text{Mg}^{2+} \cdot 3\text{H}_2\text{O}$ cationic complex system is evidently stabilized by six strong hydrogen bonds $\text{OH} \cdots \text{H}$, as also illustrated in Fig. 3. From this point of view it should be noted that the optimized structure of the $1 \cdot \text{Mg}^{2+} \cdot 3\text{H}_2\text{O}$ cationic complex species stabilized by the mentioned hydrogen bonds (Fig. 3) is apparently much more real than the two optimized structures of the complex $1 \cdot \text{Mg}^{2+}$ (Figs. 1, 2). This fact confirms the calculated binding energy of the $1 \cdot \text{Mg}^{2+} \cdot 3\text{H}_2\text{O}$ complex species ($-1,785.7 \text{ kJ mol}^{-1}$),

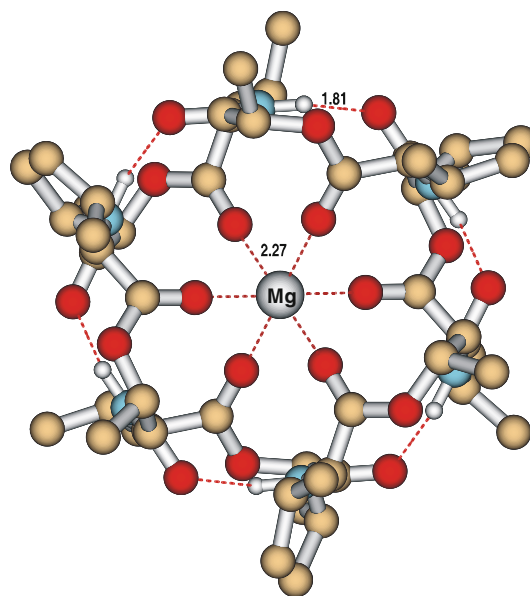


Fig. 2 The DFT optimized “symmetrical” structure of the $1 \cdot \text{Mg}^{2+}$ complex [B3LYP/6-31G(d)] (hydrogen atoms are omitted for clarity except for those taking part in internal hydrogen bonds $\text{NH} \cdots \text{O}$)

which is substantially higher than the above-given binding energies corresponding to the “asymmetrical” and “symmetrical” structures of the $1 \cdot \text{Mg}^{2+}$ complex.

Experimental

Cesium dicarbollylcobaltate (CsDCC) was purchased from Katchem, Řež, Czech Republic. A nitrobenzene solution of HDCC [7] was prepared from CsDCC by the procedure described in Ref. [17]. Equilibration of the nitrobenzene solution of HDCC with a stoichiometric amount of $\text{Sr}(\text{OH})_2$, which was dissolved in an aqueous solution of $\text{Sr}(\text{NO}_3)_2$ (0.2 M), yielded the corresponding $\text{Sr}(\text{DCC})_2$ 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 $^{85}\text{Sr}^{2+}$ (DuPont, Belgium) was of standard radiochemical purity.

The extraction experiments were conducted in 10 cm^3 glass test-tubes with polyethylene stoppers. An aqueous solution of $\text{Mg}(\text{NO}_3)_2$ (2 cm^3) with a concentration in the range 1×10^{-3} to $1 \times 10^{-2} \text{ M}$ and micro amounts of $^{85}\text{Sr}^{2+}$ were added to 2 cm^3 of a nitrobenzene solution of **1** and $\text{Sr}(\text{DCC})_2$, whose initial concentrations also varied from 1×10^{-3} to $1 \times 10^{-2} \text{ M}$ (in all experiments, the initial concentration of **1** in nitrobenzene, $C_1^{\text{in, nb}}$, was equal to the initial concentration of $\text{Sr}(\text{DCC})_2$ in this medium, $C_{\text{Sr}(\text{DCC})_2}^{\text{in, nb}}$). The test-tubes filled with these solutions were shaken for 2 h at $25 \pm 1 \text{ }^\circ\text{C}$, using a laboratory

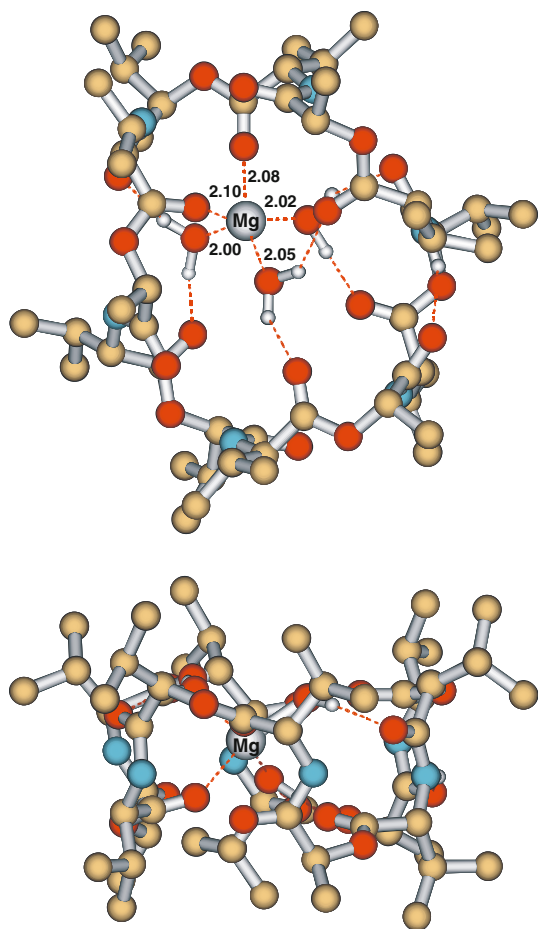


Fig. 3 Two projections of the DFT optimized structure of the $1\text{-Mg}^{2+}\cdot 3\text{H}_2\text{O}$ complex [B3LYP/6-31G(d)] (hydrogen atoms are omitted for clarity except for those of three water molecules and those taking part in internal hydrogen bonds $\text{NH}\cdots\text{O}$)

shaker. The phases were then separated by centrifugation. Samples (1 cm^3) were then taken from each phase and their γ -activities were measured using a well-type NaI(Tl) scintillation detector connected to a NK 350 γ -analyzer (Gamma, Budapest, Hungary). The equilibrium distribution ratios of strontium, D_{Sr} , were determined as the ratios of the measured radioactivities of $^{85}\text{Sr}^{2+}$ in the nitrobenzene and aqueous samples.

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