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ORIGINAL PAPER

A combined experimental and theoretical study on the complexation of the ammonium cation 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–ammonium complex in nitrobenzene saturated with water was determined. Further, the structure of the resulting complex was derived by means of density functional theory (DFT) calculations.

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

Introduction

Valinomycin (**1**, Scheme 1), an antibiotic dodecadepsipeptide, was discovered in *Streptomyces fulvissimus* cultures [1]. It consists of three identical fragments (L-valine-D-α-hydroxyisovaleric acid-D-valine-L-lactic acid), and its 36-membered ring contains six amide and six ester bonds (Scheme 1). Valinomycin was one of the first recognized ion carriers, or ionophores. The ability of valinomycin to carry ions across a membrane is primarily due to its formation of a molecular complex with them, and secondarily to the lipophilic nature of the outer rim of its depsipeptide ring, which secures its embedding into the membrane [2, 3]. Valinomycin is a highly selective ligand for potassium ions over sodium ions, and it is also a powerful uncoupler of oxidative phosphorylation in mitochondria [4].

Recently, experimental evidences for a valinomycin–proton complex and for some unusual divalent cation complexes of valinomycin have been reported [5, 6]. Further, the theoretical structures of the valinomycin complexes with Li^+, K^+, and Mg^{2+} have been solved [7–9]. On the other hand, in the current work, the stability constant of the valinomycin–ammonium complex $\left(\mathbf{1}\cdot NH_4^+\right)$ in nitrobenzene saturated with water is determined. Moreover, by applying quantum mechanical calculations, the most probable structure of the $\mathbf{1}\cdot NH_4^+$ cationic complex species is predicted.

Results and discussion

Extraction experiments

Previous results [5, 10–12] indicated that the two-phase water–NH_4Cl/nitrobenzene–NaDCC–**1** extraction system (see "Experimental"), chosen for determination of the stability constant of the cationic complex $\mathbf{1}\cdot NH_4^+$ 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.

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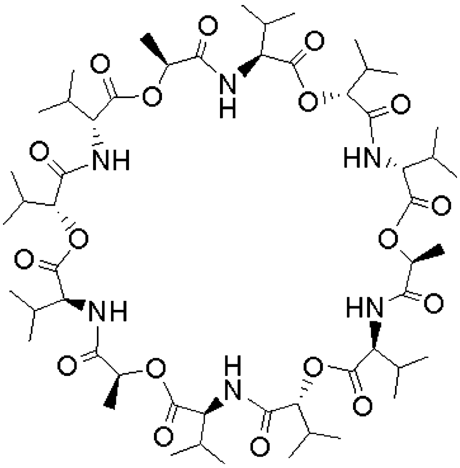

Scheme 1

$$\mathrm{NH}_4^+(\mathrm{aq}) + \mathbf{1}\cdot\mathrm{Na}^+(\mathrm{nb}) \rightleftarrows \mathbf{1}\cdot\mathrm{NH}_4^+(\mathrm{nb}) + \mathrm{Na}^+(\mathrm{aq});\quad K_{\mathrm{ex}}\left(\mathrm{NH}_4^+, \mathbf{1}\cdot\mathrm{Na}^+\right) \tag{1}$$

$$K_{\mathrm{ex}}\left(\mathrm{NH}_4^+, \mathbf{1}\cdot\mathrm{Na}^+\right) = \frac{[\mathbf{1}\cdot\mathrm{NH}_4^+]_{\mathrm{nb}}[\mathrm{Na}^+]_{\mathrm{aq}}}{[\mathrm{NH}_4^+]_{\mathrm{aq}}[\mathbf{1}\cdot\mathrm{Na}^+]_{\mathrm{nb}}} \tag{2}$$

It is necessary to emphasize that **1** is a considerably hydrophobic ligand, practically present in the nitrobenzene phase only, where this ligand forms—with NH_4^+ and Na^+—the very stable complexes $\mathbf{1}\cdot\mathrm{NH}_4^+$ and $\mathbf{1}\cdot\mathrm{Na}^+$.

Taking into account the conditions of electroneutrality in the organic and aqueous phases of the system under study, the mass balances of NH_4^+ and Na^+ ions at equal volumes of the nitrobenzene and aqueous phases, as well as the measured equilibrium distribution ratio of sodium, $D_{\mathrm{Na}} = [\mathbf{1}\cdot\mathrm{Na}^+]_{\mathrm{nb}}/[\mathrm{Na}^+]_{\mathrm{aq}}$, combined with Eq. 2, we get the final expression for the above-mentioned extraction constant (Eq. 3); $C_{\mathrm{NH_4Cl}}^{\mathrm{in,aq}}$ is the initial concentration of $\mathrm{NH_4Cl}$ in the aqueous phase and $C_{\mathrm{NaDCC}}^{\mathrm{in,nb}}$ denotes the initial concentration of NaDCC in the organic phase of the system under consideration.

$$K_{\mathrm{ex}}\left(\mathrm{NH}_4^+, \mathbf{1}\cdot\mathrm{Na}^+\right) = \frac{1}{D_{\mathrm{Na}}}\,\frac{C_{\mathrm{NaDCC}}^{\mathrm{in,nb}}}{(1+D_{\mathrm{Na}})C_{\mathrm{NH_4Cl}}^{\mathrm{in,aq}} - C_{\mathrm{NaDCC}}^{\mathrm{in,nb}}} \tag{3}$$

Table 1 Experimental data concerning the determination of $\log K_{\mathrm{ex}}(\mathrm{NH}_4^+, \mathbf{1}\cdot\mathrm{Na}^+)$ on the basis of Eq. 3

$C_{\mathrm{NH_4Cl}}^{\mathrm{in,aq}}$ (M)	$C_{\mathrm{NaDCC}}^{\mathrm{in,nb}}$ (M)	D_{Na}	$\log K_{\mathrm{ex}}$ $(\mathrm{NH}_4^+, \mathbf{1}\cdot\mathrm{Na}^+)$
1.0×10^{-3}	1.0×10^{-3}	0.030	3.0
2.5×10^{-3}	2.5×10^{-3}	0.028	3.1
5.0×10^{-3}	5.0×10^{-3}	0.031	3.0
7.5×10^{-3}	7.5×10^{-3}	0.028	3.1
1.0×10^{-2}	1.0×10^{-2}	0.034	2.9

From the extraction experiments and γ-activity measurements by using Eq. 3, the following value of the constant $K_{\mathrm{ex}}(\mathrm{NH}_4^+, \mathbf{1}\cdot\mathrm{Na}^+)$ was determined as $\log K_{\mathrm{ex}}(\mathrm{NH}_4^+, \mathbf{1}\cdot\mathrm{Na}^+) = 3.0 \pm 0.1$ (see Table 1). Furthermore, with respect to Refs. [5, 10–12], for the exchange extraction constant $K_{\mathrm{ex}}(\mathrm{NH}_4^+, \mathrm{Na}^+)$ corresponding to the equilibrium $\mathrm{NH}_4^+(\mathrm{aq}) + \mathrm{Na}^+(\mathrm{nb}) \rightleftarrows \mathrm{NH}_4^+(\mathrm{nb}) + \mathrm{Na}^+(\mathrm{aq})$ and for the extraction constant $K_{\mathrm{ex}}(\mathrm{NH}_4^+, \mathbf{1}\cdot\mathrm{Na}^+)$ defined above, as well as for the stability constants of the complexes $\mathbf{1}\cdot\mathrm{Na}^+$ and $\mathbf{1}\cdot\mathrm{NH}_4^+$ in nitrobenzene saturated with water, denoted by β_{nb} $(\mathbf{1}\cdot\mathrm{Na}^+)$ and β_{nb} $(\mathbf{1}\cdot\mathrm{NH}_4^+)$, one obtains Eq. 4.

$$\begin{aligned}\log\beta_{\mathrm{nb}}(\mathbf{1}\cdot\mathrm{NH}_4^+) = {} & \log\beta_{\mathrm{nb}}(\mathbf{1}\cdot\mathrm{Na}^+)\\ & + \log K_{\mathrm{ex}}\left(\mathrm{NH}_4^+, \mathbf{1}\cdot\mathrm{Na}^+\right)\\ & - \log K_{\mathrm{ex}}\left(\mathrm{NH}_4^+, \mathrm{Na}^+\right)\end{aligned} \tag{4}$$

Using the value $K_{\mathrm{ex}}(\mathrm{NH}_4^+, \mathrm{Na}^+) = 1.3$ inferred from Ref. [13], the constant $K_{\mathrm{ex}}(\mathrm{NH}_4^+, \mathbf{1}\cdot\mathrm{Na}^+)$ given above, log β_{nb} $(\mathbf{1}\cdot\mathrm{Na}^+) = 6.7$ [14], and applying Eq. 4, we obtain the stability constant of the $\mathbf{1}\cdot\mathrm{NH}_4^+$ complex in water-saturated nitrobenzene as $\log\beta_{\mathrm{nb}}(\mathbf{1}\cdot\mathrm{NH}_4^+) = 8.4 \pm 0.1$. It means that in this medium the stability constant of the considered $\mathbf{1}\cdot\mathrm{NH}_4^+$ cationic complex species is somewhat higher than that of the complex $\mathbf{1}\cdot\mathrm{Na}^+$ evaluated previously [14].

Quantum mechanical calculations

The quantum mechanical calculations were carried out at the density functional theory (DFT, B3LYP functional) level using the Gaussian 03 suite of programs [15]. The 6-31G(d) basis set was used and the optimizations were unconstrained. Although a possible influence of a polar solvent on the detailed structures of **1** and $\mathbf{1}\cdot\mathrm{NH}_4^+$ could be imagined, our quantum calculations, performed in an analogous way, showed very good agreement between experiment and theory [16–21].

In the model calculations, we optimized the molecular geometry of the parent valinomycin ligand **1** and its complex with NH_4^+. The optimized structure of free **1** having C_3 symmetry, very much like that ingeniously derived by early researchers from their experimental data [22–30], has been presented in our previous paper [7].

In Fig. 1, the structure obtained by the DFT optimization of the $\mathbf{1}\cdot\mathrm{NH}_4^+$ complex is depicted together with the lengths of the corresponding hydrogen bonds (in Å). Compared to free ligand **1**, the valinomycin part of the complex $\mathbf{1}\cdot\mathrm{NH}_4^+$ is only slightly distorted so that its structure is still close to C_3 symmetry. The NH_4^+ ion, placed inside the cage formed by the valinomycin ligand **1**, is predominantly bound by three strong hydrogen bonds to three ester carbonyl oxygen atoms (1.89, 1.89, 1.95 Å) and,

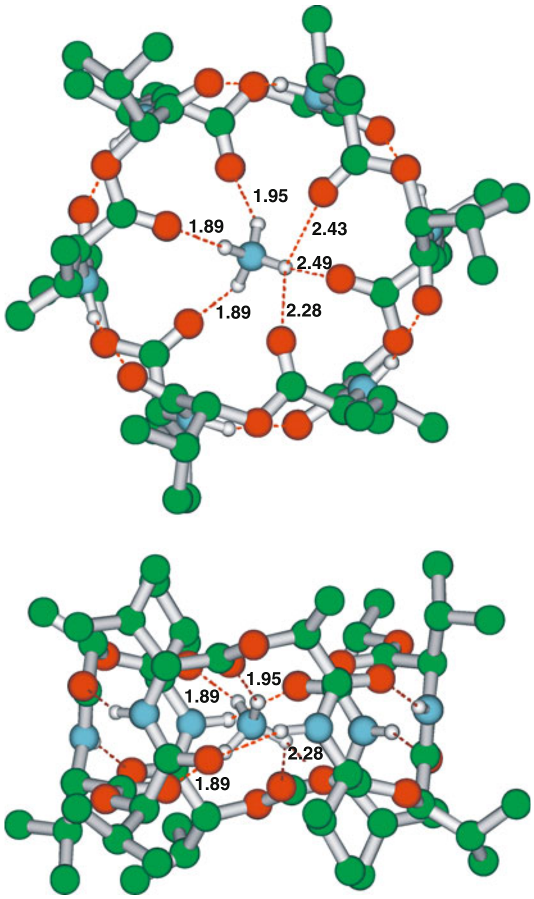

Fig. 1 Two projections of the DFT-optimized structure of the $\mathbf{1} \cdot NH_4^+$ complex [B3LYP/6-31G(d)]; hydrogen atoms omitted for clarity except those of NH_4^+ and six hydrogens taking part in six internal hydrogen bonds between the nearest peptide units

besides, the last hydrogen of NH_4^+ is bound by somewhat weaker hydrogen bonds to the remaining three ester C=O groups (2.43, 2.49, 2.28 Å). The position of the considered "central" NH_4^+ cation in the valinomycin cage is thus slightly eccentric.

Finally, the calculated binding energy of the complex $\mathbf{1} \cdot NH_4^+$ is -343.6 kJ mol^{-1}, which confirms the very high stability of this complex species.

Experimental

Valinomycin (**1**) was purchased from Fluka, Buchs, Switzerland. Cesium dicarbollylcobaltate (CsDCC) was synthesized by means of the method described by Hawthorne et al. [31]. A nitrobenzene solution of hydrogen dicarbollylcobaltate (HDCC) [10] was prepared from CsDCC by the procedure published elsewhere [32]. The other chemicals used (Lachema, Brno, Czech Republic) were of reagent grade purity. 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. The radionuclide $^{22}Na^+$ (DuPont, Belgium) was of standard radiochemical purity.

The extraction experiments were performed in 10-cm^3 glass test tubes with polyethylene stoppers: 2 cm^3 of an aqueous solution of NH_4Cl 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, $C_1^{\mathrm{in,nb}}$, was equal to the initial concentration of NaDCC in this medium, $C_{\mathrm{NaDCC}}^{\mathrm{in,nb}}$). 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 by using a well-type NaI(Tl) scintillation detector connected to a γ-analyzer NK 350 (Gamma, Budapest, Hungary).

The equilibrium distribution ratios of sodium, D_{Na}, were determined as the ratios of the measured radioactivities of $^{22}Na^+$ in the nitrobenzene and aqueous samples.

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