Monatshefte für Chemie 137, 157–161 (2006)
DOI 10.1007/s00706-005-0422-8

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

Experimental Evidence for a Valinomycin – Proton Complex

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Received June 17, 2005; accepted (revised) July 5, 2005
Published online January 20, 2006

Summary. From extraction experiments and γ-activity measurements, the extraction constant corresponding to the equilibrium $H^+(aq) + NaL^+(nb) = HL^+(nb) + Na^+(aq)$ taking place in the two-phase water-nitrobenzene system (L = valinomycin, aq = aqueous phase, nb = nitrobenzene phase) was evaluated as $\log K_{ex}(H^+, NaL^+) = -1.1 \pm 0.1$. Further, the stability constant of the valinomycin-proton complex in nitrobenzene saturated with water was calculated as $\log \beta_{nb}(HL^+) = 5.3 \pm 0.1$. Finally, the stability constants of complexes of some univalent cations with valinomycin were summarized and discussed.

Keywords. Antibiotics; Macrocycles; Protonation; Stability constant; Valinomycin.

Introduction

The antibiotic valinomycin was discovered in cultures *Streptomyces fulvissimus* [1]. It is a macrocyclic depsipeptide exhibiting threefold symmetry (see Fig. 1) [2]. It forms complexes with alkali metal ions. The stabilities of these complexes show a pronounced dependence on the ion radius [2–5]. Because of the lipophilic outer envelope of the complex, valinomycin enables univalent cations to be transported across membranes of cells and cell organelles [3], and consequently, it is a powerful decoupler of oxidative phosphorylation in mitochondria [6]. It also gives rise to ion-selective membrane potentials at bilayer lipid membranes [3]. The very large difference in the stabilities of the potassium and the sodium complexes is the cause of the high potassium selectivity of the valinomycin-based ion-selective electrode [7].

Up to now, a complex species of the proton with valinomycin has not been proved. In the present communication, the stability of protonated valinomycin in nitrobenzene saturated with water was evaluated.

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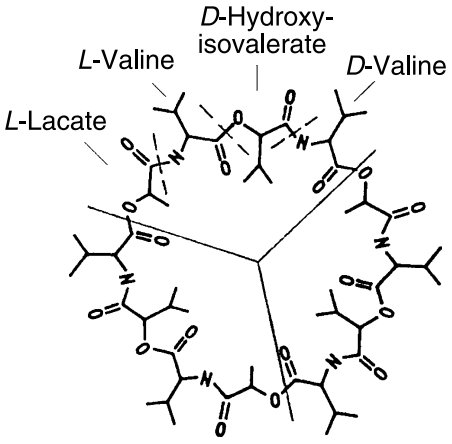

Fig. 1. Formula of valinomycin

Results and Discussion

With regard to the previous results [9, 10] the two-phase water-HCl/nitrobenzene-Na*DCC* extraction system can be described by the following equilibrium (1) with the corresponding exchange extraction constant $K_{ex}(H^+, Na^+)$; aq and nb denote the presence of the species in the aqueous and nitrobenzene phases.

$$H^+(aq) + Na^+(nb) = H^+(nb) + Na^+(aq); \qquad K_{ex}(H^+, Na^+) \tag{1}$$

For the constant $K_{ex}(H^+, Na^+)$ it can be written [9] as shown by Eq. (2) where $K^i_{H^+}$ and $K^i_{Na^+}$ are the individual extraction constants for H^+ and Na^+ in the water-nitrobenzene system [9].

$$\log K_{ex}(H^+, Na^+) = \log K^i_{H^+} - \log K^i_{Na^+} \tag{2}$$

Knowing $\log K^i_{H^+} = -5.7$ [9] and $\log K^i_{Na^+} = -6.0$ [9], the exchange extraction constant $K_{ex}(H^+, Na^+)$ was simply calculated from Eq. (2) as $\log K_{ex}(H^+, Na^+) = 0.3$.

In terms of previous papers [9–12], the two-phase water-HCl/nitrobenzene-Na*DCC*-*L* (*L* = valinomycin) extraction system, chosen for determination of stability of the complex HL^+, can be characterized by the main chemical equilibrium (3) to which the equilibrium extraction constant as shown by Eq. (4) corresponds.

$$H^+(aq) + NaL^+(nb) = HL^+(nb) + Na^+(aq); \qquad K_{ex}(H^+, NaL^+) \tag{3}$$

$$K_{ex}(H^+, NaL^+) = \frac{[HL^+]_{nb}[Na^+]_{aq}}{[H^+]_{aq}[NaL^+]_{nb}} \tag{4}$$

It is necessary to emphasize that valinomycin is a considerably hydrophobic ligand, practically present in the nitrobenzene phase only, where this ligand forms – with H^+ and Na^+ – the relatively stable complexes HL^+ and NaL^+.

Following the conditions of electroneutrality in the organic and aqueous phases of Eqs. (5) and (6), the mass balances of hydrogen and sodium at equal volumes of the nitrobenzene and aqueous phases of Eqs. (7) and (8), and the measured equili-

brium distribution ratio of sodium (Eq. (9)), combined with Eq. (4) the final expression for the extraction constant (Eq. (10)) is obtained.

$$[HL^+]_{nb} + [NaL^+]_{nb} = C^{in,nb}_{NaDCC} \tag{5}$$

$$[H^+]_{aq} + [Na^+]_{aq} = C^{in,aq}_{HCl} \tag{6}$$

$$[H^+]_{aq} + [HL^+]_{nb} = C^{in,aq}_{HCl} \tag{7}$$

$$[Na^+]_{aq} + [NaL^+]_{nb} = C^{in,nb}_{NaDCC} \tag{8}$$

$$D_{Na} = [NaL^+]_{nb}/[Na^+]_{aq} \tag{9}$$

$$K_{ex}(H^+, NaL^+) = \frac{1}{D_{Na}} \frac{C^{in,nb}_{NaDCC}}{(1 + D_{Na})C^{in,aq}_{HCl} - C^{in,nb}_{NaDCC}} \tag{10}$$

From the extraction experiments and γ-activity measurements by using Eq. (10), the following value of the constant $\log K_{ex}(H^+, NaL^+)$ was evaluated: $\log K_{ex}(H^+, NaL^+) = -1.1 \pm 0.1$.

Moreover, with respect to Refs. [12] and [13], for the extraction constants $K_{ex}(H^+, Na^+)$ and $K_{ex}(H^+, NaL^+)$ defined above as well as for the stability constants of the complexes NaL^+ and HL^+ in nitrobenzene saturated with water, denoted by $\beta_{nb}(NaL^+)$ and $\beta_{nb}(HL^+)$, one gets Eq. (11).

$$\log \beta_{nb}(HL^+) = \log \beta_{nb}(NaL^+) + \log K_{ex}(H^+, NaL^+) - \log K_{ex}(H^+, Na^+) \tag{11}$$

Using the constants $\log K_{ex}(H^+, Na^+)$ and $\log K_{ex}(H^+, NaL^+)$ given above, the value $\log \beta_{nb}(NaL^+) = 6.7$ [14], and applying Eq. (11), we obtain the stability constant of the valinomycin-proton complex in nitrobenzene saturated with water at 25°C as $\log \beta_{nb}(HL^+) = 5.3 \pm 0.1$.

The stability constants of complexes of some univalent cations with valinomycin in methanol, acetonitrile, and nitrobenzene saturated with water are listed with the corresponding references in Table 1. From here it follows that in the media under consideration, valinomycin forms the most stable complex with Rb^+ and the weakest one with Na^+ or H^+ (in nitrobenzene saturated with water) – the stability of the potassium complex is only slightly lower than that of the rubidium complex. Furthermore, the stability constants of the same metal complexes increase in the series methanol < acetonitrile < nitrobenzene saturated with water. Since the forma-

Table 1. Logarithms of stability constants of some univalent cations with valinomycin in methanol (a), acetonitrile (b), and nitrobenzene saturated with water (c)

Medium	H^+	Na^+	K^+	Rb^+	Cs^+
a	–	0.7[a]	4.5[a]	4.8[a]	3.9[a]
b	–	2.6[b]	6.7[b]	6.9[b]	6.0[b]
c	5.3[c]	6.7[d]	10.4[e]	11.7[f]	10.1[g]

[a] Ref. [4]; [b] Ref. [15]; [c] this work; [d] Ref. [14]; [e] Ref. [16]; [f] Ref. [17]; [g] Ref. [13]

tion of an arbitrary complex is assumed to be accompanied by competition for the respective cation between solvent molecules and ligand bonding sites, the present results are obviously a consequence of the decrease in basicity and solvating power of the medium in the order methanol > acetonitrile > nitrobenzene saturated with water.

Experimental

Valinomycin was supplied by Merck, Darmstadt, Germany. Cs dicarbollylcobaltate (Cs*DCC*) was synthesized in the Institute of Inorganic Chemistry, Řež, Czech Republic, using the method published by *Hawthorne et al.* [8]. The other chemicals used (Lachema, Brno, Czech Republic) were of reagent grade purity. The radionuclide ^{22}Na (DuPont, Belgium) was of standard radiochemical purity.

In order to obtain the solution of H*DCC* in nitrobenzene, the solution of Cs*DCC* in this medium (0.2 *M*) was equilibrated twice with equal volumes of 15% (*v*/*v*) *n*-propanol in diluted H_2SO_4 (about 1 *M*). H_2SO_4 and *n*-propanol were removed from the organic phase by a ten-fold equilibration with an equal volume of a solution of H_2SO_4 in distilled H_2O (about 1 *M*) followed by two equilibrations with distilled H_2O. The concentration of H*DCC* in nitrobenzene was determined by neutralization titration (NaOH, bromocresol green) after a ten-fold dilution with ethanol or acetone. The equilibration of the nitrobenzene solution of H*DCC* with stoichiometric NaOH, which was dissolved in an aqueous solution of NaCl (0.2 *M*), yields the corresponding Na*DCC* solution in nitrobenzene.

The extraction experiments were carried out in 10 cm^3 glass test-tubes covered with polyethylene stoppers: 2 cm^3 of the aqueous HCl (1×10^{-4}–1×10^{-3} *M*) and microamounts of ^{22}Na were added to 2 cm^3 of the nitrobenzene solution of valinomycin and Na*DCC*, whose initial concentrations varied also from 1×10^{-4} to 1×10^{-3} *M* (in all experiments, the initial concentration of valinomycin in nitrobenzene, $C_L^{in,nb}$, was always equal to the initial concentration of Na*DCC* 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. Under these conditions, an equilibrium in the system under study was established after approximately 15 min. Then the phases were separated by centrifugation (2 min, 2500 rpm). 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 single channel 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.

Acknowledgements

The present work was supported by the Czech Ministry of Education, Youth and Sports, Projects Nos. MSM 4977751303 and MSM 6046137307.

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