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Electrochemical Reduction of Monovalent Cation Complexes of Macrocyclic Ionophores. II. Valinomycin and Macrotetrolide Complexes

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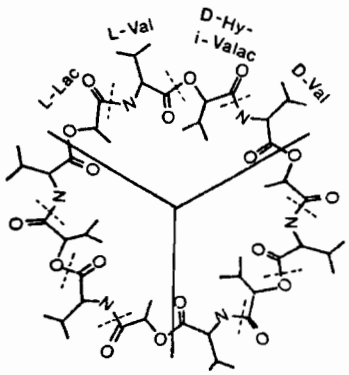
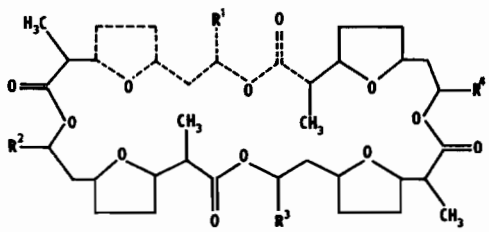
The stability and selectivity of complex formation of natural macrocyclic ionophores with alkali metal ions and monovalent thallium ion was studied by polarography. With valinomycin both stability constants and homogeneous dissociation rate constants were determined from polarographic kinetic currents. The macrotetrolides gave diffusion controlled currents. The stability of their complexes increases with the degree of substitution from nonactin to trinactin. The properties of natural ionophores were compared with those of crown polyethers. The selectivity of complex formation of valinomycin almost coincides with its effect on the increase of the conductivity of bilayer lipid membranes.

Introduction

The most important naturally occurring ionophores are the 36-membered depsipeptid valinomycin (formula I) [1, 2] and the macrotetrolides [3–5] which are tetralactones of nonactinic acid and its derivatives (actine antibiotics, formula II).



I



II

$R^1=R^2=R^3=R^4=CH_3$	nonactin
$R^1=R^2=R^3=CH_3$, $R^4=C_2H_5$	monactin
$R^1=R^2=CH_3$, $R^3=R^4=C_2H_5$	dinactin
$R^1=CH_3$, $R^2=R^3=R^4=C_2H_5$	trinactin
$R^1=R^2=R^3=R^4=C_2H_5$	tetranactin

Among macrocyclic ion carriers valinomycin shows one of the highest selectivities for potassium with respect to sodium ions. The structure of the free substance as well as of its complexes, their conformation both in solid state and in solution of different permitivity, the complex stability and formation rate have been investigated by means of various physical and physicochemical methods [6–18].

The macrotetrolides selectively form complexes with alkali metal ions [13, 14, 18, 19]. The conformation of free molecules and their complexes in solution has not been elucidated in detail while it is known that considerable conformation changes in the ligand ring result owing to complex formation [20–22].

In two preliminary communications the polarographic determination of stability as well as the rate of formation of valinomycin complexes [15] and of stability of macrotetrolide complexes [13] with monovalent cations was described. In this paper we present a detailed treatment of all experimental results obtained and full discussion of their stability in comparison with that of cyclic polyethers described in the preceding communication [23].

Experimental

Materials

Valinomycin was kindly supplied by Professor V. T. Ivanov of the Shemyakin Institute of Bio-

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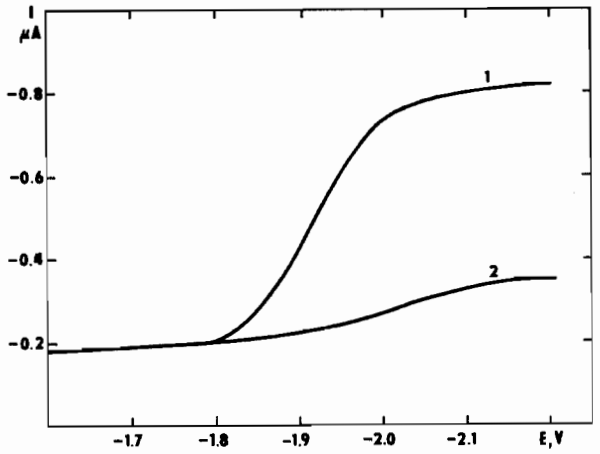

Fig. 1. Polarographic reduction wave of 1.8×10^{-4} *M* KSCN, curve 1; polarographic reduction wave of 1.5×10^{-4} *M* KSCN in the presence of valinomycin in the concentration 7.7×10^{-4} *M*, curve 2; base electrolyte 0.05 *M* TBAP (tetrabutylammonium perchlorate) in acetonitrile, $t_1 = 0.7$ sec, t = 23 °C.

organic Chemistry, Academy of Sciences of U.S.S.R., Moscow; the macrotetrolides by Professor V. Prelog, Swiss Federal Polytechnic, Zurich, and nonactin by Dr. K. Thurm, Central Institute of Microbiology and Experimental Therapy, Academy of Sciences of G.D.R., Jena. Other chemicals were analytical grade products of Lachema Brno, Czechoslovakia, or were prepared in the laboratory. Acetonitrile was purified using the recently described method [24].

Apparatus

The polarograms were recorded with the polarographs OH-102 and OH-105 (Radelkis, Budapest, Hungary). The dependence of the momentaneous current of reduction of the complexes at the mercury dropping electrode (the 'I–t curve') was measured in the potentiostatic arrangement by means of the Wenking LB 75L Potentiostat (G. Bank Elektronik, FRG) and the X–Y plotter BAK 4T, Aritma, Prague.

Results

Valinomycin

In the presence of an excess of valinomycin the polarographic waves of monovalent cations decrease quite considerably (Fig. 1). The new limiting current in the case of potassium, rubidium and cesium ions is almost independent of the height of mercury column. The momentaneous current is proportional to $t^{2/3}$. At higher valinomycin concentrations the limiting current is inversely proportional to the square root of valinomycin concentration. We expect, therefore, that the decrease of the limiting current is due to slow dissociation of the complex

$$(MV)^+ \underset{k_f}{\overset{k_d}{\rightleftharpoons}} M^+ + V \qquad (1)$$

The analysis of the polarographic waves is based on the following assumptions [25]:

i) Under equilibrium conditions the complex prevails considerably over the free metal ions ($K_{MV} \cdot c_V = (k_f/k_d) \cdot c_V \gg 1$).

ii) Only free metal ions are reduced. The direct reduction of the complex does not interfere with this process.

iii) The chemical reactions (1) are, indeed, not fast enough as to preserve the equilibrium of this process but they are still sufficiently fast so that a steady state of formation of free metal ions by (1) and their diffusion to the electrode is established.

iv) The chemical rate equation and the time of electrolysis have such values that the resulting limiting current $\langle I_l \rangle$ due to the reduction of free metal ions at the electrode is considerably larger (at least four times) than the hypothetical limiting diffusion current corresponding to the very small concentration of free metal ions. Again, it must be much smaller than the limiting diffusion current due to the reduction of the complex (for average limiting currents, $\langle I_l \rangle < 1/10 < \langle I_d(MV) \rangle$), see Fig. 1.

v) The electrode potential has a sufficiently negative value so that all free metal ions arriving at the electrode are reduced (condition of limiting current). Thus, for the distance from the electrode x = 0, $[M^+] = 0$.

Under these conditions, the average limiting 'kinetic' current is given by equation

$$\langle I_l \rangle = j_l \langle A \rangle = 0.51 F m^{2/3} t^{2/3} C_{MV} \sqrt{(k_d D_M / K_{MV} c_V)} \qquad (2)$$

where $\langle A \rangle$ is the average area of the dropping electrode, m the flow-rate of mercury (g s^{-1}) and t_l the drop-time.

If the inequality for the value of $\langle I_l \rangle$ in the condition iv is not fulfilled, diffusion of $(MV)^+$ must be taken into account. Then equation (2) will be modified to the form

$$\frac{\langle I_l \rangle}{\langle I_d(MV) \rangle - \langle I_l \rangle} = 0.886 \sqrt{(k_d D_M t_l / K_{MV} c_v D_{MV})} \qquad (3)$$

From equation (2), resp. (3), the rate constant k_d will be determined if K_{MV} is known.

If the electrode reaction of the free metal is reversible then a simple equation holds for the half-wave potential of the kinetic wave [25],

$$E_{1/2} = E^\theta + (RT/2F) \ln(D_{M,Hg}/D_{MV}) -$$

$$- (RT/F) \ln(K_{MV} c_V) + (RT/F) \ln [\langle I_d(MV) \rangle / \langle I_l \rangle] \qquad (4)$$

where E^θ is the formal potential of the system metal ion/metal dissolved as amalgam, $D_{M,Hg}$ the diffusion

TABLE I. Stability Constants K_{st} and Rate Constants of Formation k_f and Dissociation k_d of Valinomycin Complexes with Alkali Metal Ions and Monovalent Thallium in Acetonitrile, 0.05 *M* Tetrabutylammonium Perchlorate, t = 23 °C.

	Na^+	K^+	Rb^+	Cs^+	Tl^+
$\lg K_{st}$	2.6	6.7	6.9	6.0	5.9
$\lg k_f$	–	8.5	8.6	8.2	–
$\lg k_d$	–	1.8	1.7	2.2	–

coefficient of the metal in mercury and $\langle I_d(MV)\rangle$ is the average diffusion current of the complex which can be determined by means of the Ilkovič equation. The diffusion coefficient of the complex is calculated on the basis of an assumed size of the complex, resulting in an error of about ±10% in the values of stability constants. By means of this equation the stability constants of the complexes have been determined using the half-wave potential of the kinetic wave.

As could be seen from the I–t curve the limiting current of the thallium(I) complex was controlled solely by diffusion. Thus the stability constant in this case was determined in the same way as in Part I [23]. The stability of the sodium complex is quite low so that no appreciable shift of the half-wave-potential could be observed. The complex stability in this case was determined by means of the exchange reaction

$$Na^+ + (TlV)^+ \rightleftharpoons (NaV)^+ + Tl^+ \qquad (5)$$

In large excess of Na^+ the half-wave potential of the wave of the thallium(I) complex was shifted to more positive potentials since the originally free valinomycin was bound from a large part in the sodium complex. This shift is related to the stability constant of the sodium complex

$$\Delta E_{1/2} = (RT/F)\ln[(K_{TlV}/K_{NaV})(c_V - c_{Tl})/(c_{Na} - c_V + c_{Tl})] \qquad (6)$$

The stability constants of the complexes are listed in Table I.

On the basis of eq. (3) the rate constants of homogeneous reaction of formation and dissociation of potassium, rubidium and cesium complexes have been determined (Table I).

Macrotetrolides

The presence of macrotetrolides nonactin, monactin, dinactin and trinactin in solutions containing alkali metal cations results in a shift of half-wave potentials of reduction waves to more negative potentials depending on concentration of the ligand and in some decrease of the limiting current. The limiting current remained, however, under diffusion control. The stability constants were determined in the same way as in Part I [23] (see Table II). On the basis of the diffusion coefficients estimated from the limiting diffusion currents the Stokes radii of the complexes were calculated (Table III).

TABLE II. Stability Constants and Macrotetrolide Complexes K_{st} with Alkali Metal Ions in Acetonitrile, 0.025 *M* Tetrabutylammonium Perchlorate, t = 22 °C.

	Nonactin	Monactin	Dinactin	Trinactin
Na^+	4.0	4.3	4.4	–
K^+	4.4	4.8	5.2	5.4
Rb^+	3.9	–	–	–
Cs^+	2.6	–	–	–

TABLE III. Stokes Radii of Macrotetrolide Complexes with Potassium in Acetonitrile, 0.025 *M* Tetrabutylammonium Perchlorate, t = 22 °C.

	Nonactin	Monactin	Dinactin	Trinactin
r_{St}/nm	0.399	0.438	0.637	0.781

Discussion of Equilibria of Complex Formation

Valinomycin

The standard Gibbs energy of complex formation of the ion I with the ligand X in a solvent α

$$\Delta G^{o,\alpha}_{f,I} = -RT \ln K_{IX} \qquad (7)$$

is given by the difference of the standard Gibbs energy of transfer of the ion from vacuum into the cavity of the ligand present in that particular solvent and of the standard Gibbs energy of solvation in the same solvent,

$$\Delta G^{o,\alpha}_{f,I} = \Delta G^{o,vac\rightarrow X}_{tr,I} - \Delta G^{o,\alpha}_{s,I} \qquad (8)$$

In acetonitrile valinomycin forms the most stable complex with rubidium and, among the cations investigated in the present paper, the weakest one with sodium (Table I). The stability of the potassium complex is only slightly lower than that of the rubidium complex.

Let us take as a measure of solvation of alkali metal ions in acetonitrile the values of Gibbs energies of solvation of alkali metal chlorides [26]. With the help of these data and of equations (7) and

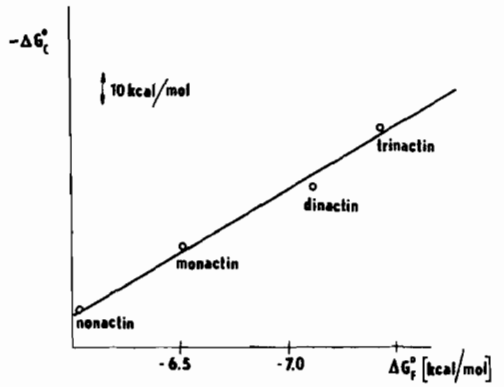

Fig. 2. Correlation curve between the Gibbs free energy values ΔG_c° (calculated by EHT method) and the experimentally found values of Gibbs free energy ΔG_F°.

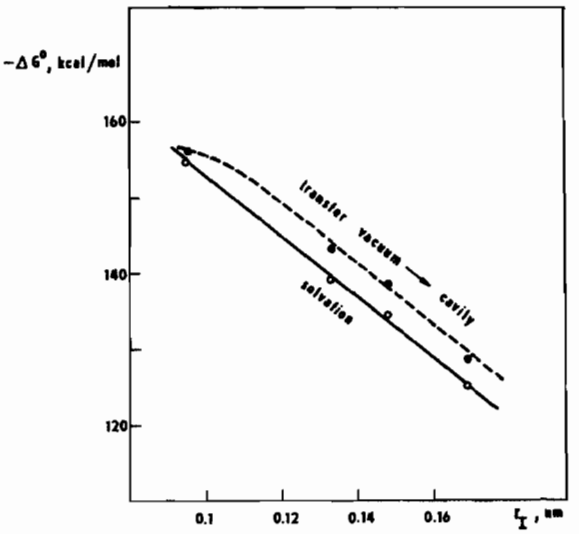

Fig. 3. The dependence of standard Gibbs energy values $-\Delta G^\circ$ of solvation and of cation transfer from vacuum into the valinomycin cavity on alkali metal ionic radii.

(8) we obtain the standard Gibbs energies of transfer of the cation from vacuum into the cavity of the ligand (the values have a constant additive term which is the standard Gibbs energy of solvation of chloride). In Fig. 3 these values have been plotted together with the standard Gibbs energies of solvation of alkali metal chlorides as a function of ion radius. Obviously the interaction of K^+, Rb^+ and Cs^+ with the ligand cavity follows approximately the same line as the interaction with the solvent so that no selectivity is observed in complex formation. On the other hand there is a conspicuous deviation for Na^+ which causes the notorious K^+–Na^+ selectivity effect. The ion radius of desolvated sodium ion is smaller than the radius of the cavity. Thus the contraction of the cavity can occur and can increase the repulsion of binding sites in the ligand which results in the lower stability of complex [27]. Although the radius of Tl^+ is almost the same as that of K^+ the stability of the complex is about one order of magnitude lower. The binding sites in the valinomycin molecule are carbonyl oxygens of ester groups with lower affinity for Tl^+ than for K^+ [12, 15, 19]. Obviously the ion radius is not the only factor affecting the stability of these 'inclusion' complexes; important influence is also exerted by the electronic structure of the ions under the valence shell affecting the nature of the bond, *etc.*

Macrotetrolides

The stability of alkali metal complexes increases in the series nonactin, monactin, dinactin and trinactin (see Table II). An attempt was made to correlate the increase in stability in the homologous series by means of the calculation of electron shift on binding sites as an effect of substitution. Since these molecules consist of four identical structural units the contributions of one quarter of the molecule (see in formula II the dotted part of molecule) were calculated by means of the EHT method (Extended Hückel Theory) [14]. Only electrostatic interactions in the multipoint field of the molecule were taken into account while the other contributions were neglected. The cation is approximated as a point charge. The calculated standard Gibbs energy changes $\Delta G^\circ_{MV,C}$ and the experimentally found standard Gibbs energy changes of complex formation $\Delta G^\circ_{MV,F}$ were correlated in Fig. 2. The resulting correlation justifies the approximation of the calculation. Upon successive substitution the electron density on the binding ether oxygens (of tetrahydrofuran rings and carbonyl oxygen atoms of ester groups) is increased which immediately enhances the strength of ion-induced dipole bond and in this way also the complex stability.

In this case a complete desolvation of cation in the complex is to be expected. The ligand molecules are flexible and can easily accommodate cations of different size. This explains a not very pronounced selectivity of macrotetrolides for alkali metal ions in contrast to valinomycin.

Joint Discussion of Part I and II

It seemed worthwhile to compare the results of complex formation of complexes of synthetically prepared cyclic polyethers and of macrotetrolides and of valinomycin which are natural products.

The macrocyclic compounds studied in Part I [23] and II are electroneutral molecules which act as selective cation carriers in thin and thick membranes and uncouple oxidative phosphorylation even though the same effect requires a polyether concentration by

three orders of magnitude higher than that of valinomycin [28, 29]. All these substances form 'inclusion' complexes of ion–dipole character. The difference between cyclic polyethers with a maximum number of ring atoms of 24 and the other macrocyclic compounds appears first with the conformation of the complexes. Among the crown polyethers the arrangement of oxygen atoms is almost coplanar and the central cation is able to feed-up the coordination bonds in the direction perpendicular to the plane of oxygen atoms. The spectroscopic studies of the complexes in the solid state have proved the coordination of solvent molecules or anions [30]. Thus, the central cation is only partially desolvated which explains the rather low selectivity of these ligands in the series of alkali metal cations. The formation is in the first place ruled by the 'competition strength' between the binding sites of the ligand and the solvent molecules of the primary solvation sheath of the cation.

In the case of the 30-membered cyclic polyether, of the macrotetrolides and of valinomycin the central ion is completely desolvated and perfectly separated from the solvent. Here the difference in standard Gibbs energies of solvation of individual cations plays a major role. Besides this the steric conditions are also important, particularly the size of ligand cavity with respect to the radius of the central cation and the rigidity of resulting complex.

One of the important results of the polarographic study of the alkali metal complexes with macrocyclic ligands was the detection of the kinetic control of reduction of several valinomycin complexes. While in all other cases the electrode processes are governed by diffusion, in this case the rate-determining step is the dissociation of the complex.

The formation of cyclic polyether complexes is a rapid reaction. The highest values were found in the case of alkali metal complexes of dibenzo-30-crown-10 and its dimethyl derivative where the rate constant is nearly independent of cation radius [6]. In this case the formation rate constant approaches the value corresponding to a diffusion controlled reaction ($k_f \approx 10^9$ l mol^{-1} s^{-1}). For this case the mechanism of a rapid successive substitution of solvent molecules from the solvation sheath of the cation by binding sites of the ligand has been proposed [6, 31]. This process, with participation of a sufficiently flexible ligand with easy transition from one configuration to another, has such a low activation energy that the diffusion of reacting particles determines the overall rate.

With slower reactions a successive substitution of solvent molecules is also expected but, in a certain situation, several bonding sites are occupied simultaneously which results in an increase of the activation energy and in retardation of the process. In the case of formation of the valinomycin complex with alkali metal ions a conformation intermediate stage is

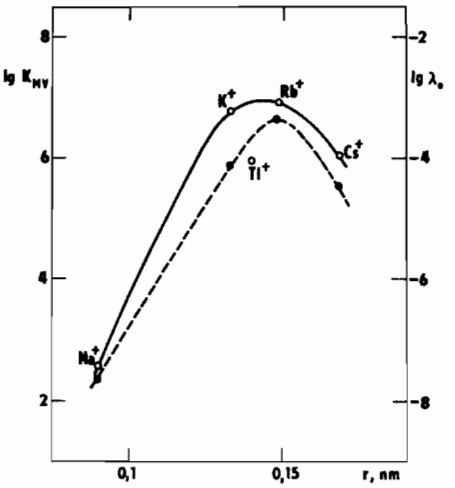

Fig. 4. The dependence of stability constants K_{MV} of valinomycin complexes (full curve), and of the conductivity λ^o of bilayer lipid membranes modified by valinomycin in the presence of ions in water solutions [31] (dotted curve) on ionic radii of alkali metal ions and monovalent thallium ion.

assumed [6] so that several solvent molecules are substituted at once and the remaining molecules of the solvation sheath are replaced in a subsequent conformation change of monomolecular character. According to Grell *et al.* [6] the first step is not ion-selective, being equally rapid for alkali metal cations. Only the following conformation change of this intermediate form to the final product shows a distinct ion-selectivity. The results obtained by polarography in the present paper with rate constants of valinomycin complex formation with potassium, rubidium and cesium ions of the order of 10^8 l mol^{-1} s^{-1} are close to the diffusion limit which indicates that the rapid equilibrium between the original and intermediate stage is characterized by constants of the order of 10^{-1} l mol^{-1}.

The membrane-carrier properties of valinomycin and of 30-membered polyether have been compared [27]. Although the ion transfer mediated by valinomycin is highly selective, it is slower than the transfer by means of the cyclic polyether. This phenomenon can be, indeed, caused by the kinetic control in the case of valinomycin in contrast with the diffusion control in the case of the polyether.

It would seem of interest to compare the conductance of bilayer lipid membrane modified by valinomycin in the presence of various alkali metal cations [32] with the complex stabilities in acetonitrile. Fig. 4 shows that the course of the dependence of both these quantities on cation radii is almost identical.

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