ABSORPTION POTENTIALS AT HEPTANE-WATER INTERFACE IN PRESENCE OF VALINOMYCIN

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The presence of the cyclic antibiotic valinomycin (VM) [1] makes the bimolecular lipid membrane selectively permeable to the potassium ion [2]. The transmembrane potential in a concentration cell was thoroughly investigated in [3, 4] with the aid of specially constructed electrodes made selective for the potassium ion by means of VM. The relationship between the potential of the electrochemical concentration cell and the logarithm of the potassium ion activity is linear with a slope of 58 mV. We tested this result in experiments with a cell simulating the membrane

$$\begin{array}{c|c|c|c|c|c|c|c|c} \mathrm{Hg} & \mathrm{Hg_2Cl_2} & \mathrm{KCl} & \mathrm{KCl} & \mathrm{VM} & \mathrm{KCl} & \mathrm{KCl} & \mathrm{Hg_2Cl_2} & \mathrm{Hg,} \\ & & \mathrm{sat.} & C_1 & \mathrm{heptane} & C_2 & \mathrm{sat.} & & \\ & & & \mathrm{(water)} & & \mathrm{(water)} & & & \end{array} \qquad (1)$$

where the concentration C_1 was constant (10^{-2} M KCl) and the concentration C_2 varied, as indicated in Fig. 1a.

It was of interest to find the relationship between the potential difference at the heptane-water interface and the K^+ ion concentration in a cell containing only one water-nonwater boundary. The investigated cell can be represented in traditional electrochemical notation in the following way:

$$\begin{array}{ccccccccccc} 1 & & 2 & & 3 & & 4 & & 5 & & 6 \\ \mathrm{Au} & | & \mathrm{air} & | & \mathrm{heptane} & | & \mathrm{KCl,\ VM} & | & \mathrm{KCl} & | & \mathrm{Hg_2Cl_2} \mid \mathrm{Hg} \\ & & & & & & \mathrm{(water)} & & \mathrm{sat.\ (water)} & & \\ & \varphi_{12} & & \varphi_{23} & & \varphi_{34} & & \varphi_{45} & & \varphi_{56} & \end{array} \qquad (2)$$

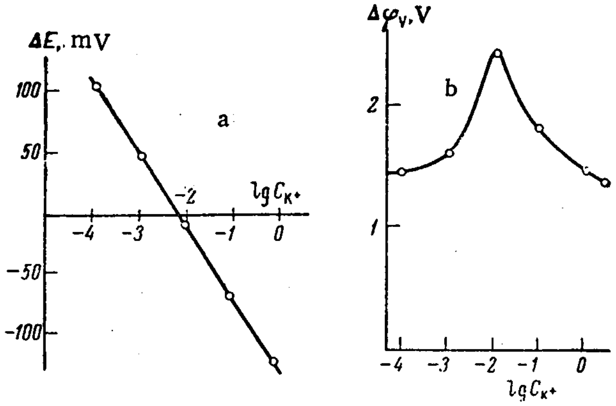

Fig. 1. Dependence of transmembrane and interphase potentials on K^+ ion concentration in presence of 10^{-6} M VM. a) In cell (1); b) in cell (2).

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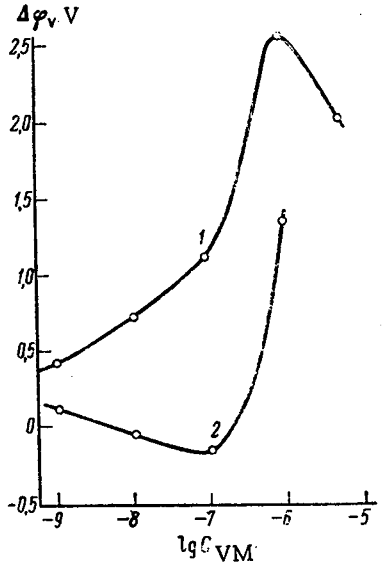

Fig. 2

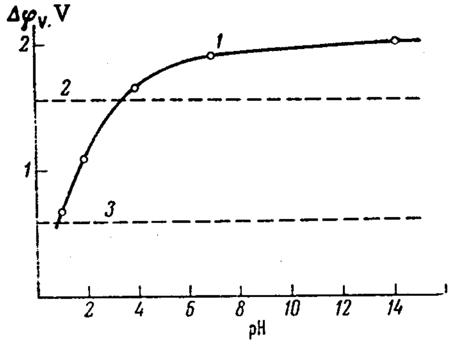

Fig. 3

Fig. 2. Dependence of interphase potential at heptane-water interface on VM concentration in presence of: 1) 10^{-2} M KCl; 2) 10^{-2}M NaCl.

Fig. 3. Effect of pH on change of Volta potential at heptane-water boundary in presence of: 1) 10^{-2} M KCl + 10^{-6} M VM + 10^{-3} M $C_6H_{13}COOH$; 2) 10^{-2} M KCl + 10^{-6} M VM; 3) 10^{-2} M KCl.

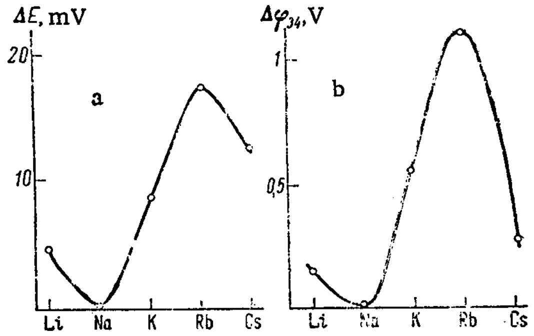

Fig. 4. Transmembrane and interphase potentials in relation to nature of cation with [MeCl] = 10^{-4} in the presence of 10^{-6} M VM. a) Biionic potentials of bilayer phospholipid membranes from data of [2]; b) interphase potentials relative to Na^+ ion potential.

With the method used we were able to measure the change in Volta potential between a gold reference electrode and the heptane in relation to the composition of the water phase by the vibrating-capacitor method. In the apparatus used there was a Pyrex glass cell with a bowl in which the heptane was on top of the water phase. A vibrating gold reference electrode was placed above the heptane phase. The reference electrode on the water-phase side was a calomel electrode connected with the cell by a salt bridge containing saturated KCl solution.

All the water solutions were made up with twice-distilled water and twice-recrystallized salts. The heptane was normal standard grade and was contained in the same vessel as the water phase where it was kept for 36 h (or less, if the mixture had been agitated on a shaker); in this time equilibrium was established. The valinomycin was introduced into the prepared system in the form of a highly concentrated heptane or alcohol solution. The salt composition of the water phase after equilibrium with the heptane was established can be regarded as practically constant, as was indicated by the slope of the emf cell (1) in relation to KCl concentration, which corresponded with the theoretical slope.

If the four potential discontinuities φ_{12}, φ_{23}, φ_{45}, and φ_{56} remain constant when the composition of the water phase changes the entire observed effect can be attributed to the change in the potential discontinuity φ_{34} at the heptane-water interface. Special experiments on the system

$$\mathrm{Au}\ \underset{\varphi_{12}}{\Big|}\ \mathrm{air}\ \underset{\varphi_{23}}{\Big|}\ \begin{matrix}\mathrm{K\,(VM)^+\ \ Cl^-}\\ \mathrm{VM,heptane}\end{matrix}\Big|\begin{matrix}\mathrm{heptane}\\ \mathrm{AgCl}\end{matrix}\Big|\ \mathrm{Ag} \qquad (3)$$

showed that the Volta potential remained almost constant when the composition of the heptane phase changed. The heptane phase consisted of heptane separated from a heptane-water solution system in which equilibrium had been established. Since the potential discontinuity φ_{12} was constant the constancy of the Volta potential would indicate the constancy of φ_{23}. We actually observed a slight shift of the Ag potential towards more negative values (by 10-11 mV when the KCl concentration in the water phase was changed by an order). This was probably due to an increase in the Cl^- content in heptane.

The constancy of the discontinuity φ_{23} means that there is no change in Galvani potential at the heptane-air interface. In addition, the absence of adsorption at the heptane–air boundary was confirmed by measurements of the surface tension of heptane at the air boundary. Figure 1b shows the change in Volta potential φ_V in cell (2) with change in the K^+ ion concentration in the water phase in the presence of 10^{-6} M VM. Figure 2 shows $\Delta\varphi_V$ in relation to VM concentration in 10^{-2} M KCl and 10^{-2} M NaCl solutions. It is clear that the Volta potentials are higher in the presence of the K^+ ion. An increase in VM concentration from 10^{-7} to 10^{-6} M led to a sharp increase in the Volta potential; at VM concentrations of more than 10^{-6} M in the presence of K^+ ions there was some decrease in the measured value of $\Delta\varphi_V$.

In the presence of VM and K^+ ions and also at higher VM and Na^+ ion concentrations the Volta potential values were shifted into the region of positive values. The shift was usually 0.8 to 3 V and depended in an undeterminable manner on the choice of the VM sample used to prepare the initial VM solution, which was then added to the heptane-water system. The shift, however, was reproducible within the series of solutions prepared from the same VM sample.

The addition of enanthic acid to the heptane phase when the pH of the water solution exceeded the pK of enanthic acid increased the Volta potential due to the presence of VM, and when $pH < pK$ reduced it to the values characteristic for the system in the absence of VM (Fig. 3). This undoubtedly indicates a relationship between the observed Volta potentials and adsorption effects at the heptane-water interface. The presence of the organic anion probably facilitates the charging of the heptane by the $K(VM)^+$ complex, whereas the strongly adsorbed undissociated enanthic acid molecules displace $K(VM)^+$ from the interface.

It is of particular interest to compare the Volta potentials measured at the same concentrations of alkali metal chlorides. It is assumed that VM forms complexes, soluble in the heptane phase, mainly with K^+ and Rb^+ ions [5-7]. Hence, the distribution coefficients and, consequently, the interphase potentials will be different for different cations. Figure 4b shows the values of $\Delta\varphi_{34}$, relative to φ_{34} in the case of Na^+, for Li^+, Na^+, K^+, Rb^+, and Cs^+ cations for $[MeCl] = 10^{-2}$ M and $[VM] = 10^{-6}$ M. The Volta potentials for systems containing K^+ and Rb^+ ions are highest. Figure 4a shows the corresponding biionic potentials of phospholipid membranes from the data of [2]. Although the behavior of the potential difference relative to cation atomic radius in the two cases is very similar, the absolute values in the case of cells with the same interface are almost two orders higher.

A comparison of Figs. 1a and 4a with Figs. 1b and 4b indicates the nonadditivity of the potential at the heptane-water interface when a transmembrane potential arises. A possible explanation of this nonadditivity will be discussed in our next paper.

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