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Electrically Silent Anion Transport through Bilayer Lipid Membrane Induced by Tributyltin and Triethyllead

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Summary. The method of the measurement of the nonelectrogenic fluxes of hydrogen (or hydroxyl) ions (J_H) based on the local proton gradients formation in the unstirred layers near a bilayer lipid membrane (BLM) is applied for recording the nonelectrogenic anion/OH^- exchange on BLM induced by tributyltin (TBT) and a novel carrier (Hager, A., Moser, I., & Berthold, W. 1987. *Z. Naturforsch.*, **42C:**1116–1120), triethyllead (TEL). This method has been used previously for measuring the cation fluxes through BLM. TBT and TEL are shown to be equally efficient in the induction of Cl^-/OH^- exchange. J_H induced by TBT is constant at $4 < pH < 7$. J_H decreases at $pH < 4$ and $pH > 7$. Both ionophores have a transport sequence: $I^- > Br^- > Cl^- > F^-$. The quantitative measurements reveal that TEL better discriminates these four anions than TBT. It is concluded that this method may prove helpful in a search and study of anion/OH^--exchangers isolated from natural membranes.

Key Words anion transport · tributyltin · triethyllead · bilayer lipid membrane · anion selectivity

Introduction

The ion-selective ionophores give an important tool for the study of the physiological role of ions. At present nonelectrogenic ionophores for potassium, sodium and calcium ions (for instance nigericin, monencin and A23187 (Westley, 1982, 1983; Pressman, 1976)) are available as well as electrogenic ionophore for potassium and calcium (for instance valinomycin and EXT 1001 (Pressman, 1976; Hinds & Vincenzi, 1985; Deber & Hsu, 1986)). In contrast to cation ionophores there are only three nonelectrogenic ionophores for anions with similar ion selectivity: tributyltin (Selwyn et al., 1970), triethyllead (Hager, Moser & Berthold, 1987) and Amberlite LA-2 (Gutknecht, James & Tosteson, 1978; Gutknecht & Walter, 1979) and one electrogenic Cl^--ionophore benzyl viologen (Kim et al., 1982) which can operate only at low redox potentials.

The present work deals with two anion/OH^--exchangers: tributyltin (TBT) and a novel carrier, triethyllead (TEL). TBT catalyzes the electrically silent anion transport through artificial (Tosteson & Wieth, 1979*b*; Ivanov, Petrov & Antonov, 1978) and natural (Selwyn et al., 1970; Tosteson & Wieth, 1979*a*; Antonov & Ivanov, 1975) membranes. TBT has a transport sequence: $I^- > Br^- > Cl^- > F^-$ (Tosteson & Wieth, 1979*a*,*b*). It was shown that the ion flux is proportional to the TBT concentration thereby providing 1 : 1 anion-ionophore stoichiometry (Tosteson & Wieth, 1979*b*). TEL is a potent Cl^-/OH^--exchanger for the membranes of chloroplasts (Hager et al., 1987). The action of TEL on a model membranes has not been studied so far.

In the present work I have measured the nonelectrogenic hydroxyl ion flux induced by the difference in I^-, Br^-, Cl^- and F^- concentrations at opposite sides of the planar bilayer lipid membrane (BLM) in the presence of TBT and TEL. The experimental procedure is based on the phenomenon of the formation of local ion concentration gradients in the course of transmembrane ion fluxes in the unstirred layers near the BLM. This method was applied previously to measure the hydrogen ion fluxes induced by the gradient of cations in the presence of cation/H^+-exchangers (Antonenko & Yaguzhinsky, 1983*a*,*b*, 1988). The data obtained are discussed in terms of a model of Cl^-/OH^--exchange which is similar to the model of K^+/H^+-exchange proposed by Pressman and coworkers (1967) for the nigericin operation.

Materials and Methods

BLM is formed on a Teflon partition 0.4 mm in diameter, by a conventional method (Mueller et al., 1963). A membrane-forming solution contains 20 mg phosphatidylcholine from soybeans (Sigma) and 10 mg cholesterol (Boehringer) in 1 ml of *n*-decane. The thinning of the BLM is observed both visually and by measuring its capacity. The main element of the electrical scheme is

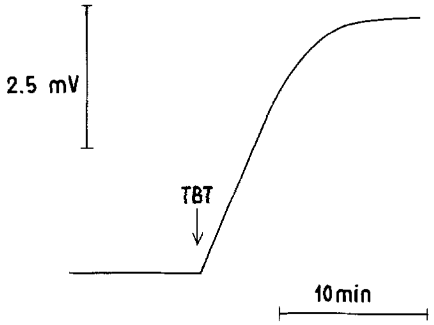

Fig. 1. The generation of the electrical potential on BLM with KCl concentration gradient (80 mM KCl against 50 mM) in the presence of TTFB (10 μM) and on addition of tributyltin (TBT, 1.6 μM). The solution was: 1 mM Tris, 1 mM MES, 1 mM β-alanine, pH 7.0. The potential had plus on the side of the BLM where the concentration of KCl was higher

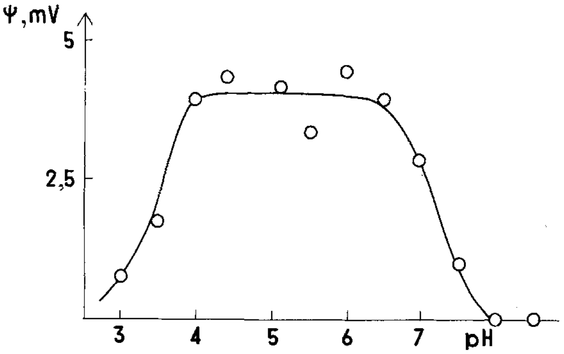

Fig. 2. pH dependence of the nonelectrogenic hydrogen ion flux J_H in the presence of TBT (0.8 μM). The conditions were as in the caption to Fig. 1

Keithley 301 amplifier. The experiments are carried out at room temperature. The hydrogen ion electroneutral flux (J_H) is measured by the method which was described earlier (Antonenko & Yaguzhinsky, 1983*a,b*). The pH gradient in the unstirred layers is determined from the difference of the electrical potentials on BLM in the presence of a protonophore in the open circuit mode. It was shown that under these conditions the J_H flux is proportional to the membrane potential (Antonenko & Yagushinsky, 1983*b*). J_H is calibrated against the potential by adding the increasing concentrations of sodium acetate at different pH values as described earlier (Antonenko & Yaguzhinsky, 1983*b*). The buffer mixture Tris, MES and β-alanine (all from Serva) slightly affected J_H values in the pH range 4.5–8.5 J_H varied from 4.7 to 5.5×10^{-11} mole H^+/cm^2sec (the average was 5×10^{-11}) under these conditions. The protonophore, tetrachlorotrifluoromethylbenzimidazole, tributyltin (Serva) and triethyllead (a generous gift of Prof. A. Hager, University of Tubingen, FRG) are added at both sides of the BLM. MES, Tris is from Serva, β-alanine from Reanal (Hungary), other chemical from Reachim (USSR).

Results

Figure 1 shows the kinetics of the generation of the electrical potential on BLM with a potassium chloride concentration gradient in the presence of the protonophore TTFB on the addition of TBT. The potential has plus on the side of the membrane where the concentration of KCl is higher. The control experiments reveal that the generation of the potential can not be accounted for by the induction of anion electrogenic permeability since the BLM conductance is not increased upon the addition of TBT in the absence of TTFB (*data not shown*).

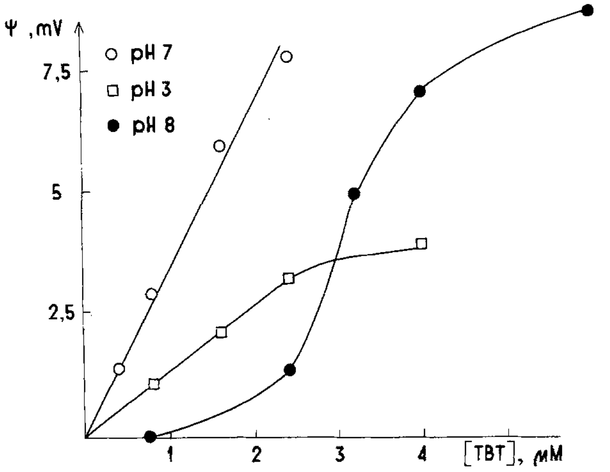

Fig. 3. The dependence of J_H on the TBT concentration. The conditions were as in the caption to Fig. 1

Figure 2 shows the pH dependence of the potential on the membrane in the presence of TBT. The ψ is a measure of the J_H flux under these conditions (*see* Materials and Methods).

It is seen that at $pH < 4$ and $pH > 7$ the J_H flux decreases and at $4 < pH < 7$ J_H is constant. At $pH = 6.5$ and $pH = 3$ the J_H flux is proportional to the TBT concentration while at $pH = 8$ this dependence has S-type form (Fig. 3). The S-type dependence is observed also at $pH = 7$ under the conditions when the KCl concentration is decreased (10 mM at one side of the membrane and 1 mM at the other).

Figure 4 shows the effect of the gradient of KCl concentration on the J_H flux. It is seen that the curves are straight lines at pH 3, 6, 5 and 8. The dependence of the J_H flux on the TEL concentrations is presented in Fig. 5. The comparison of Figs. 2 and 4 shows that at $pH = 7$ TBT and TEL induce similar J_H fluxes. The J_H flux is proportional to the TEL concentration with apparent saturation at the higher TEL concentration.

The anion selectivity measurements are performed by the previously developed method (Antonenko & Yaguzhinsky, 1988). Figure 6 shows an example of these measurements. After the potential on the BLM attained a steady-state value in the

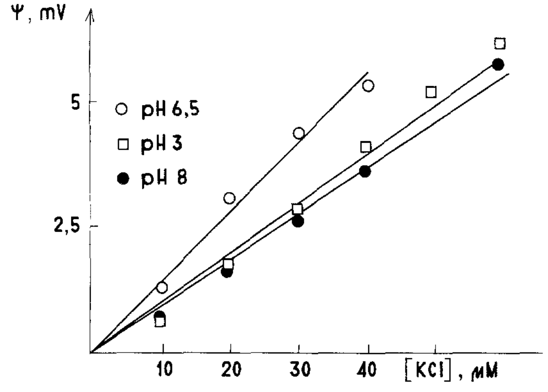

Fig. 4. The dependence of J_H on the KCl concentration at one side of the membrane. At pH 6.5 the concentration of TBT was 0.8 μM, 3.0–1.6 μM, and 8.0–2.4 μM. The conditions were as in the caption to Fig. 1 plus 50 mM KCl

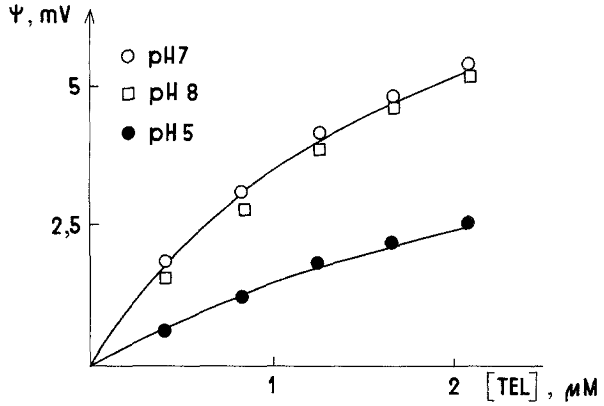

Fig. 5. The dependence of J_H on the triethyllead (TEL) concentration. The conditions were as in the caption to Fig. 1

presence of an anion/OH$^-$-exchanger and a protonophore (given the existence of a concentration gradient of the anion) the reverse gradient using the other anion is formed and increased until the BLM potential reaches zero. It was shown earlier that the ratio of transmembrane gradients of appropriate ions under the conditions of zero potential is a good measure of ion selectivity of an ionophore (Antonenko & Yaguzhinsky, 1988). It is obtained for TBT; Cl$^-$/F$^-$, 2.3; Br$^-$/Cl$^-$, 2.3; and I$^-$/Br$^-$, 3.3. This gives the following transport sequence with respect to iodine: I$^-$(1) > Br$^-$(0.3) > Cl$^-$(0.13) > F$^-$(0.06). The experiments with TEL show: Cl$^-$/F$^-$, 7; Br$^-$/Cl$^-$, 11; I$^-$/Br$^-$, 6; and the transport sequence, I$^-$(1) > Br$^-$(0.17) > Cl$^-$(0.015) > F$^-$(0.002).

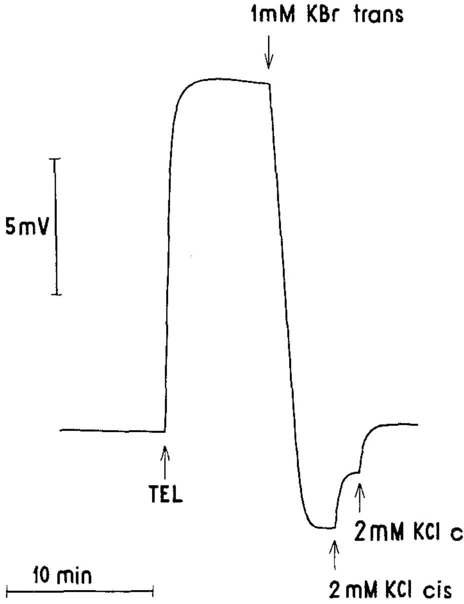

Fig. 6. An example of the measurement of anion selectivity of the anion/OH$^-$-exchanger TEL. Potential generation with KCl concentration gradient (9 mM on *cis* side of BLM) in the presence of 10 μM TTFB on the addition of 14 μM TEL. The solution was 1 mM Tris, 1 mM MES, 50 mM β-alanine, pH 6.5. After the potential on the BLM had attained a steady-state value the reverse gradient using KBr was formed until the BLM potential reached zero

Discussion

The method of the measurements of electrically silent anion fluxes used in this work is similar to the previously employed approach for measuring cation fluxes (Antonenko & Yaguzhinsky, 1983*a,b*). According to electroneutrality of the total process the unidirectional anion flux induced by the difference in anion concentration at different sides of the BLM should induce the reverse unidirectional flux of hydroxide anions (Cl$^-$/OH$^-$-exchange) or the flux of hydrogen ions in the same direction (Cl$^-$/H$^+$-simport). The hydroxide ion flux (as well as the hydrogen ion flux) should form the pH gradient in the unstirred layers of BLM in the solutions of low buffer capacity. The pH gradient is measured by the electrical potential recording in the presence of a protonophore. It was shown that under these conditions the J_H flux is proportional to the membrane potential (Antonenko & Yaguzhinsky, 1983*b*).

Figure 7 depicts the model of Cl$^-$/OH$^-$-exchange in the presence of a translocator T$^+$. The model implies the existence of equilibrium stages of Cl$^-$ and OH$^-$ binding by T$^+$ at membrane-water interfaces and rate determining stages of the translocation of TCl and TOH complexes across the membrane. It was mentioned above that the process of

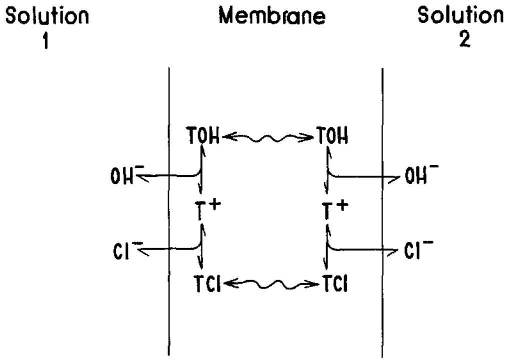

Fig. 7. Transfer of monovalent anions across the membrane by a nonelectrogenic ionophore. Solutions *1* and *2* represent different sides of the membrane, *T* is the carrier; the straight arrow represents the chemical reaction and the wavy arrow, the translocation across the membrane

Cl^-/OH^--antiport and Cl^-/H^+-simport can not be discriminated in our experiments but it is reasonable to propose that the cationic molecules TBT and TEL bind the hydroxide anion rather than the hydrogen cation. Gutknecht and coworkers (1978) discussed the mechanism of Cl^-/H^+-simport in the case of Amberlite LA-2-mediated anion transport.

The model presented here explains the pH dependence of the J_H flux induced by TBT. At pH < 4 the flux is limited by the TOH form of the carrier the concentration of which decreases with pH decrease. At pH > 4 the process is limited by the TCl form of the translocator. At $4 <$ pH < 7 the increase in the concentration of the TOH form does not alter the concentration of the TCl form in the membrane (the flux is independent of pH). At pH > 7 the increase in the TOH concentration decreases the TCl concentration and accordingly the J_H flux.

The model predicts the linear dependence of the J_H flux on the concentration of the carrier. Figure 2 shows that this is valid only for pH < 7 and low concentrations of TBT. At pH > 7 this dependence has the S-type form. I think that this peculiarity is accounted for by the behavior of the translocator, e.g., by the formation of oligomeric complexes of TCl (or/and TOH) in the alkaline range of pH, rather than by changes in the membrane structure, since the linear dependence of J_H on the concentration of TEL is observed at pH = 8.

The transport sequence of TBT $I^- > Br^- > Cl^- > F^-$ coincides with that obtained by Tosteson and Wieth (1979*b*), who measured the chloride flux across the BLM in the presence of other anions, and with the sequence determined on the erythrocyte membranes. The quantitative measurements of anion selectivity reveal that TEL can better discriminate anions, say F^- and Cl^- (seven times) than TBT (2.3 times). This observation shows that TEL has some advantages as an anion-selective ionophore for physiological experiments.

It should be said in conclusion that for the first time the measurements of the nonelectrogenic H^+/OH^--fluxes are applied to the recording of the of Cl^-/OH^--exchange process on BLM. This method may prove useful in a search and study of an anion/OH^--exchangers isolated from natural membranes. There are several anion-transporting systems in the membranes of animal cells: erythrocyte (Knauf, 1979), mitochondria (Garlid & Beavis, 1986), neutrophyl (Simonowitz & De Weer, 1986). On the other hand, the existence of anion/OH^--exchangers may be proposed in the membranes of procaryotes since cation/H^+-exchangers are wide-spread antibiotics (Westley, 1982).

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