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Generation of Potential in Lipid Bilayer Membranes as a Result of Proton-Transfer Reactions in the Unstirred Layers

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Abstract

The addition of an uncoupler in the presence of a concentration gradient of weak acids or bases (sodium acetate and ammonium chloride) leads to the generation of a potential on lipid bilayer membranes (LBM) which is positive in sign on the side of the membrane with a high concentration of sodium acetate and negative on the side with a high concentration of ammonium chloride. It is shown that the potential was caused by the pH gradient in the unstirred layers. These effects can be understood in terms of the previously described [*Science*, **182,** 1258 (1973)] model for the transfer of weak acids and bases through LBM. This system described may be useful for quantitation of permeabilities for weak acids and bases through bilayer membranes.

Key Words: Membrane permeability; unstirred layers; lipid bilayers.

Introduction

The previous studies (Gutknecht and Tosteson, 1973; Gutknecht *et al.*, 1977; Gutknecht and Walter, 1980; LeBlanc, 1971; Borisova *et al.*, 1974) contained indirect evidence for the formation of pH gradients in the unstirred layers during the transmembrane transfer of weak acids through black lipid membranes. In another series of studies (Ismailov *et al.*, 1973, 1974; Yaguzhinsky *et al.*, 1974) it was shown that potentials form across bilayer membranes in the course of oxidation–reduction reactions, which results in the formation of a hydrogen ion concentration gradient in the unstirred layers. The present work is concerned with potential generation across LBM upon the formation of transmembrane salt gradients of weak acids and bases in the presence of the

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uncoupler. Thus, the generation of an electrical potential on the membranes due to restriction of the diffusion of acids and bases through the unstirred layers has been established.

Experimental

Lipid bilayer membranes were formed by the standard technique (Yaguzhinsky *et al.*, 1974) on a 0.4-mm hole in a Teflon partition. The membrane-forming mixture contained 20 mg egg lecithin and 20 mg cholesterol per 1 ml *n*-decane. The formation of the membrane was controlled visually and also by measurement of its electric capacity. A magnetic stirrer was used for mixing solutions in the cell. Potentials were measured by means of AgCl electrodes connected with solutions by agar-agar bridges filled with 0.01 M KCl. The potential was controlled with a Keithley 301 amplifier connected to a recorder. The measurements were carried out at room temperature.

Results

The addition of ammonium chloride or sodium acetate on one side of the membrane in the presence of tetrachlorotrifluoromethylbenzimidazole (TTFB), a protonophore, results in the generation of potential difference across the membrane (Fig. 1). The resulting potentials have opposite signs for these two salts, i.e., in the case of sodium acetate the potential is positive on the side where the salt is added. In both cases the potentials are discharged on adding valinomycin. Without protonophore, the addition of the salts brings about no potential generation, the membrane conductance remaining unaltered. In these and subsequent experiments the pH of the solutions was maintained constant during the course of the experiment.

It has been shown that diffusional polarization of proton is observed in unstirred layers in the presence of TTFB in unbuffered solutions (Borisova *et al.*, 1974). This effect results in a decrease of the observed diffusional potential compared to the theoretical value. Solutions with low buffer capacity were used in our experiments (Figs. 1–4). No polarization of unstirred layers occurs under these conditions, as judged by the formation of a 58-mV potential in the presence of TTFB and application of a 10-fold difference in hydrogen ion concentration across the membrane.

Figure 2 shows the dependence of the potential on the concentration of ammonium chloride (B) and sodium acetate (A) at two buffer concentrations, 1 and 10 mM. The ionic strength was held constant by changing the potassium

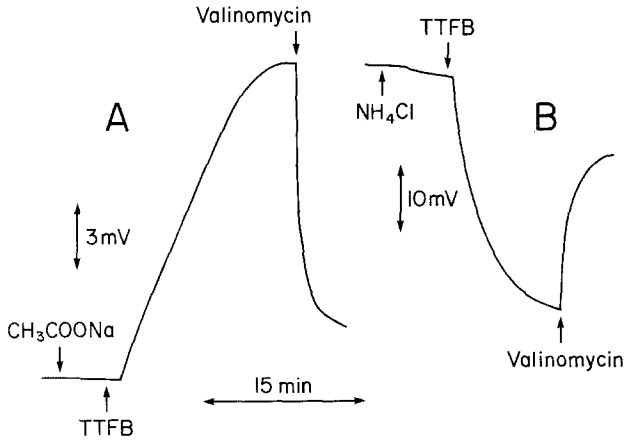

Fig. 1. Potential generation on LBM upon the addition of sodium acetate (A) and ammonium chloride (B) in the presence of TTFB. (A) concentration of CH_3COONa 6.3 mM in compartment I of the cell (in compartment II CH_3COONa was not added); TTFB 0.5×10^{-6} M and valinomycin 10^{-6} M in both compartments. Composition of the medium: 1 mM citrate, 1 mM KH_2PO_4, 1 mM succinate, 100 mM KCl, pH 6.5. (B) concentration of NH_4Cl 1 mM, only in one compartment; TTFB 10^{-5} M and valinomycin 0.5×10^{-6} M in both compartments. Composition of the medium: 1 mM HEPES, 100 mM KCl, pH 7.5.

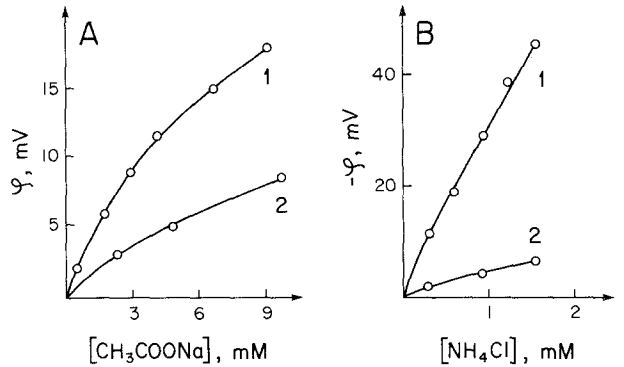

Fig. 2. Effect of the buffer capacity (curves 1 and 2) on the value of the potential across LBM at different concentration gradients for sodium acetate (A) and ammonium chloride (B). The opposite compartment of the cell was free from these salts. Composition of the medium in experiment 1: 1 mM citrate, 100 mM KCl, pH 6.5. Composition of the medium in experiment 2: 10 mM citrate, 90 mM KCl, pH 6.5. Concentration of TTFB 10^{-6} M.

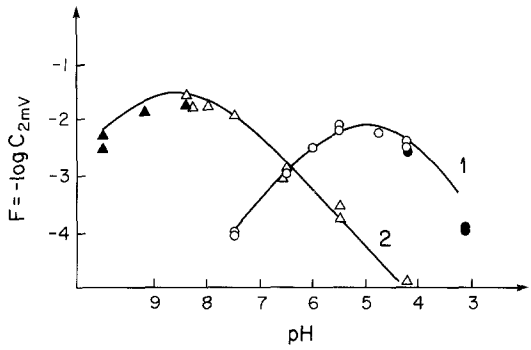

Fig. 3. Effect of pH on potential generation across LBM in the presence of the uncoupler upon formation of the concentration gradient for sodium acetate (1) and ammonium chloride (2). The ordinate shows the negative logarithm of the salt concentration gradient in micromoles resulting in a 2-mV potential. $F = -\log C_{2mV}$. (▲) Effect NH_4Cl, CCCP protonophore 2×10^{-6} M. Composition of the medium: 1 mM KH_2PO_4, 1 mM Tris, 1 mM H_3BO_3, 1 mM $NaHCO_3$, 100 mM KCl. (△) Effect NH_4Cl, TTFB protonophore 10^{-6} M. Composition of the medium: 1 mM Tris, 0.7 mM HEPES, 0.7 mM MOPS, 1 mM citrate, 1 mM succinate, 100 mM KCl. (○) Effect of CH_3COONa, same conditions. (●) Effect of CH_3COONa, TTFB 10^{-6} M. Composition of the medium: 1 mM Tris, 1 mM KH_2PO_4, 1 mM succinate, 100 mM KCl, 1 mM citrate. Solid lines indicate theoretical dependences calculated by Eq. (6).

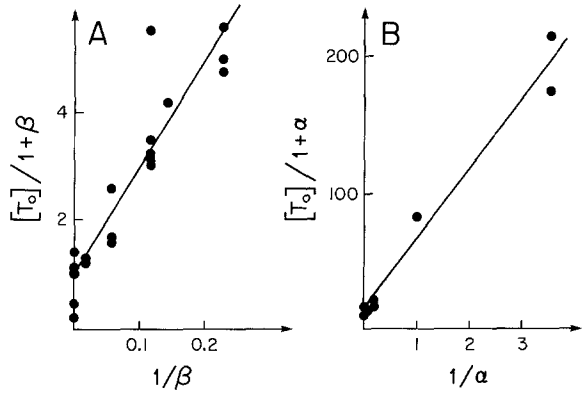

Fig. 4. The pH dependence of the formation of potential on BLM. (A) The dependence of ammonium chloride concentration $[T_0]$, μM, added from one side of the membrane on the parameter $\beta = 10^{9.24 - pH}$ in the coordinates $T_0/(1 + \beta)$ versus $1/\beta$. (B) The dependence of sodium acetate concentration $[T_0]$, μM, added from one side of the membrane on the parameter $\alpha = 10^{pH - 4.75}$ in the coordinates $T_0/1 + \alpha$ versus $1/\alpha$. The conditions are as indicated in Fig. 3. The salt concentrations at different pH were chosen to provide a potential on the membrane equal to 2 mV.

chloride concentration. In both cases increase in the buffer capacity decreases the potential.

Table I illustrates the dependence of the potential value on the salt concentration gradient. Comparison of columns 5 and 6 shows that the potential does not depend on the ratio of salt concentrations on different sides of the membranes; on the other hand, the constancy of the $\varphi/\Delta C$ ratio (column 7) allows one to believe that the potential value depends linearily on the difference between salt concentrations at low potentials. The experiments showed this regularity to be valid over a large pH range (from 3 to 10).

The pH dependence curve for potential-forming capacity, F, of the concentration gradients of salts of weak acids and bases (Fig. 3) goes through the maximum in the pH region from 4.2 to 5.5 for sodium acetate and from 7.5 to 9.2 for ammonium chloride. The ordinate represents the negative logarithm of the salt concentration gradient in micromoles at which the potential on the LBM is 2 mV. The value of the salt concentration (on the opposite side of the membrane the salt concentration is zero in these experiments) was determined by plotting the potential against the concentration at a given pH value and by extrapolation to $\varphi = 2$ mV. At pH 8.3 carbonylcyanide *m*-chlorophenylhydra-

Table I. Dependence of the Potential Across LBM (in the Presence of TTFB) on the Concentration Gradient of Sodium Acetate and Ammonium Chloride[a]

Number of addition	C_1, mM	C_2, mM	$C_1 - C_2$, mM	C_1/C_2	φ, mV	$K_1\varphi/C_1 - C_2$,[b] $K_2\varphi/C_1 - C_2$
			Sodium acetate			
1	0	0.0025	−0.0025	0	0.0	—
2	0.25	0.0025	0.25	100	1.8	1.00
3	0.63	0.0025	0.63	250	3.9	0.87
4	1.00	0.0025	1.00	400	6.0	0.83
5	1.25	0.0025	1.25	500	7.2	0.80
6	1.25	0.25	1.00	5	5.7	0.79
7	1.25	0.63	0.63	2	3.6	0.80
8	1.25	1.00	0.25	1.25	1.8	1.00
9	1.63	1.00	0.63	1.63	3.6	0.80
			Ammonium chloride			
1	0	0.0009	0.0009	0	0.0	—
2	0.094	0.0009	0.093	100	3.9	1.00
3	0.188	0.0009	0.187	200	7.2	0.92
4	0.281	0.0009	0.280	300	9.6	0.81
5	0.281	0.095	0.187	3	6.0	0.78
6	0.281	0.188	0.094	1.5	3.0	0.78
7	0.281	0.282	−0.0009	1.0	0.6	—
8	0.375	0.282	0.093	1.33	3.0	0.78

[a] C_1 and C_2 are the concentrations of the corresponding salts in the two compartments of the cell on different sides of LBM. Concentration of TTFB 10^{-6} M. Composition of the medium: Tris, 1 mM; HEPES, 0.7 mM; MOPS, 0.7 mM; citrate, 1 mM; succinate, 1 mM; KCl, 100 mM. Sodium acetate, pH 6.0; ammonium chloride, pH 8.0.

[b] K_1 is for sodium acetate, and K_2 is for amonium chloride.

zone (CCCP) was substituted for the TTFB protonophore (Borisova *et al.*, 1974; LeBlanc, 1971). From Fig. 3 it can be seen that substitution of the protonophore does not influence the magnitude of the effect.

Discussion

When the opposite sides of the LBM are exposed to different concentrations of sodium acetate or ammonium chloride, the addition of protonophore results in potential generation. This indicates that there must be a pH gradient across the membrane. Since the experiment was carried out in weakly buffered solutions without altering the pH, the observed potential must be due to a change in the pH that has occured spontaneously in the unstirred layers under the experimental conditions. This is supported by the fact that the potential value decreases as the buffer capacity of the medium increases (Fig. 2).

The data in Table I indicate that the potential value (in the first approximation) depends linearly on the difference in salt concentrations on opposite sides of the membrane. It is not altered when the ratio between salt concentrations changes as long as the difference in concentrations is constant. This shows that the effect of potential generation is controlled, according to Fick's law, by a diffusional flux of acids and bases through the unstirred layers and the membrane. The value of the flux, J, is determined by the concentration gradient of the substance, $\Delta C/\Delta x$, and at constant Δx by the difference of concentrations, Δc: $J = D\ (\Delta C/\Delta x) = P\Delta C$, where D is the diffusion coefficient and P is permeability.

The previous model for the weak acid transport through the LBM has been based on data on the diffusion of radioactively labeled compounds (Gutknecht and Tosteson, 1973). This model (see Fig. 5) explains the difference between the signs of potentials across the LBM seen in our experiments upon imposing the concentration gradients of sodium acetate and ammonium chloride: in the first case the acid form of the acid–base pair, CH_3COOH, is transferred through the membrane; in the second it is the base form, NH_3. The total flux, J_t, of the neutral form of the TH acid and anion T^-

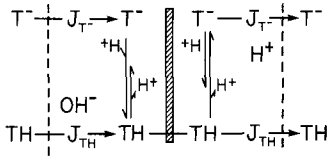

Fig. 5. Model of the transport of weak acid TH through lipid bilayer membrane (M) and unstirred layers (UL).

can be described by the equation (Gutknecht and Tosteson, 1973)

$$1/J_t = \frac{1}{P_{T^-}^{UL}[T^-] + P_{TH}^{UL}[TH]} + \frac{1}{P_{TH}^{M}[TH]} \tag{1}$$

where $P_{T^-}^{UL}$ and P_{TH}^{UL} are the permeabilities through the unstirred layer for T^- and TH, respectively, and P_{TH}^{M} is the membrane permeability for TH. The total flux of weak acid through the unstirred layers and the membrane can be conveniently considered as the sum of two independent fluxes:

$$J_{TH}: \quad J_{TH}^{UL_1} \rightarrow J_{TH}^{M} \rightarrow J_{TH}^{UL_2}$$

$$J_{T^-}: \quad J_{T^-}^{UL_1} \xrightarrow{+H^+} J_{TH}^{M} \xrightarrow{-H^+} J_{T^-}^{UL_2}$$

If the pH values of the solutions which are in contact with both sides of the membrane are the same, J_{TH} would not contribute to the change of pH in the unstirred layers. The flux J_{TH} is described by the equation.

$$\frac{1}{J_{TH}} = \frac{1}{P_{TH}^{M}[TH]} + \frac{1}{P_{TH}^{UL}[TH]} \tag{2}$$

to obtain the equation for J_{T^-} the flux J_{TH} [Eq. (2)] should be substracted from the total flux J_t [Eq. (1)]; this gives the equation

$$\frac{1}{J_{T^-}} = \left(\frac{P_{TH}^{UL} + P_{TH}^{M}}{P_{TH}^{M}}\right)\left\{\frac{1}{P_{TH}^{M}[TH]} + \left(\frac{1}{P_{T^-}^{UL}} + \frac{P_{TH}^{UL}}{P_{T^-}^{UL} \cdot P_{TH}^{M}}\right)\frac{1}{[T^-]}\right\} \tag{3}$$

With the assumptions $P_{TH}^{M} \gg P_{TH}^{UL}$ and $P_{TH}^{UL} \simeq P_{T^-}^{UL}$ (Walter and Gutknecht, 1981) Eq. three can be written in a simple form:

$$1/J_{T^-} = \frac{1}{P_{T^-}^{UL}[T^-]} + \frac{1}{P_{TH}^{M}[TH]} \tag{4}$$

In order to check whether this model [Eqs. (1)–(4)] is adequate for our system, we studied the pH dependence of the potential-forming capacity, F, of the salt concentration gradient of weak acids and bases. For a quantitative analysis, the values of the salt concentration gradients giving rise to a 2-mV potential were compared at different pH values. Under these conditions, the pH gradient between the unstirred layer and the water phase is comparatively low.[2] Thus one can use Eq. (2), which is based on the assumption that the pH value in unstirred layers undergoes no changes during the diffusion of weak acids.

[2] $\Delta pH \simeq 2/60 \simeq 0.03$.

With the buffer capacity constant, the change in pH is a function of the flux value, J_{T^-}. According to Eq. (4) three pH ranges have been established in which the system behaves differently. In the case of sodium acetate at pH > 5.5, the effect (F) decreases with pH (Fig. 3), which corresponds to decrease in the concentration of CH_3COOH in the system. At 4.2 < pH < 5.5 the plateau in the graph (Fig. 3) can be accounted for by a slight change in the concentration of CH_3COO^-. At pH < 4.2 the effect decreases with pH due to decrease in the concentration of acetate ion.

For a quantitative estimation of the parameters in Eq. (4) we transform this equation in the following way:

$$[T_0] = [T^-] + [TH], \quad \text{where} \quad [T^-] = [T_0]\frac{\alpha}{1+\alpha}$$

$$[TH] = [T_0]\frac{1}{1+\alpha} \tag{5}$$

$$\frac{[T_0]}{1+\alpha} = \frac{J_{T^-}}{P_{TH}{}^{M}} + \frac{J_{T^-}}{P_{T^-}{}^{UL}}\frac{1}{\alpha} \tag{6}$$

where $\alpha = 10^{pH - pK_a}$, $[T_0]$ is the salt concentration on one side of the membrane[3] at which the potential (at a given pH) is equal to 2 mV, and K_a is the dissociation constant for TH. In the case of ammonium chloride, Eq. (6) is analogous, the only difference being that the parameter α is substituted for the parameter $\beta = \alpha^{-1}$. The conditions of our experiments were chosen such that the values of the potentials at different pH were constant. Since there is no significant contribution of J_{TH} to pH change in the unstirred layers (see above), the value of J_{T^-} is constant (when buffer capacity is constant). The parameters $J_{CH_3COO^-}/P_{CH_3COOH}{}^{M} = 14 \pm 5\ \mu M$ and $J_{CH_3COO^-}/J_{CH_3COO^-}{}^{UL} = 51 \pm 3\ \mu M$ were calculated from the plot $[T_0]/(1+\alpha)$ versus $1/\alpha$ (Fig. 4) for sodium acetate. For ammonium chloride Fig. 4 gives $J_{NH_4^+}/P_{NH^{4+}}{}^{UL} = 20 \pm 2\ \mu M$ and $J_{NH_4^+}/P_{NH_3}{}^{M} = 1.0 \pm 0.2\ \mu M$.

It has been shown previously that the diffusion of a substance through the unstirred layers does not depend strongly on the nature of the substance and is

[3] On the other side of the LBM $[T_0] = 0$.

[4] The difference between the fluxes of $J_{CH_3COO^-}$ and $J_{NH_4^+}$ seems to be due to differences in their diffusion coefficients in water.

usually 1×10^{-3} cm/sec (see, for instance, Orbach and Finkelstein, 1980). Hence we obtain $P_{CH_3COOH}{}^{M} = 3.6 \times 10^{-3}$ cm/sec and $J_{CH_3COO^-} = 51 \times 10^{-12}$ M cm^{-2} sec^{-1}. The value of acetate permeability through the membrane is in good agreement with the 6.6×10^{-3} cm/sec value known from the literature (Walter and Gutknecht, 1981). Assuming that $P_{NH_4^+}{}^{UL} = 1 \times 10^{-3}$ cm/sec we obtain $P_{NH_3}{}^{M} = 2 \times 10^{-2}$ cm/sec and $J_{NH_4} = 20 \times 10^{-12}$ M cm^{-2} sec^{-1}.[4] We have found no data in the literature on membrane permeability to ammonia. The results obtained may be used as an experimental approach for the determination of the permeability of weak acids and bases through the membranes. This method in some cases might be more convenient than measurement of permeabilities using radioactive compounds.

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