ON THE ADSORPTION OF PHLORETIN ONTO A BLACK LIPID MEMBRANE

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ABSTRACT The effect of uncharged, dipolar phloretin on anion and cation conductance through a black lipid membrane can be used to study its adsorption behavior. The adsorption of phloretin can be described by a Langmuir isotherm with weak dipole-dipole interaction.

INTRODUCTION

It has been known for a long time (cf., Adam, 1941) that a lipid monolayer spread at the water/air interface causes a change in Volta potential. Such a potential can be thought of as resulting from dipoles in the oriented lipid molecules and/or from lipid-induced orientation of water dipoles at the water/lipid interface.

Ultrathin ("black") lipid membranes are essentially two lipid monolayers arranged back-to-back, and are believed to exhibit similar dipole potentials. These have been invoked to explain the preferential conductance of such membranes for anions over cations of similar size and hydrophobic interaction (e.g., tetraphenylborate over tetraphenylphosphonium) (Liberman and Topaly, 1969).

Dipolar molecules incorporated in ultrathin lipid membranes have been found to modify their anion and cation conductances dramatically. McLaughlin (1973) noted that salicylamide enhances the membrane conductance for cations while depressing that for anions. He ascribed this effect to a change in the membrane dipole potential upon adsorption of the neutral compound. Szabo (1974) observed a similar effect upon incorporation of cholesterol into a monoolein membrane, although the interpretation in this case is somewhat complicated by an apparent parallel change in membrane viscosity.

Andersen et al. (1976) reported on the effect of phloretin and structurally similar compounds on the membrane conductance. Their data for the conductance of different anions and cations through phosphatidyl ethanolamine membranes are shown in Fig. 1 as a function of the aqueous concentration of phloretin. The coincidence of the four sets of data, for a membrane-soluble anion (tetraphenylborate), a membrane-soluble cation (tetraphenylarsonium), an uncoupler of oxidative phosphorylation (carbonyl cyanide-*m*-chlorophenylhydrazone), and a carrier-transported cation (K^+/nonactin), clearly indicates that a com-

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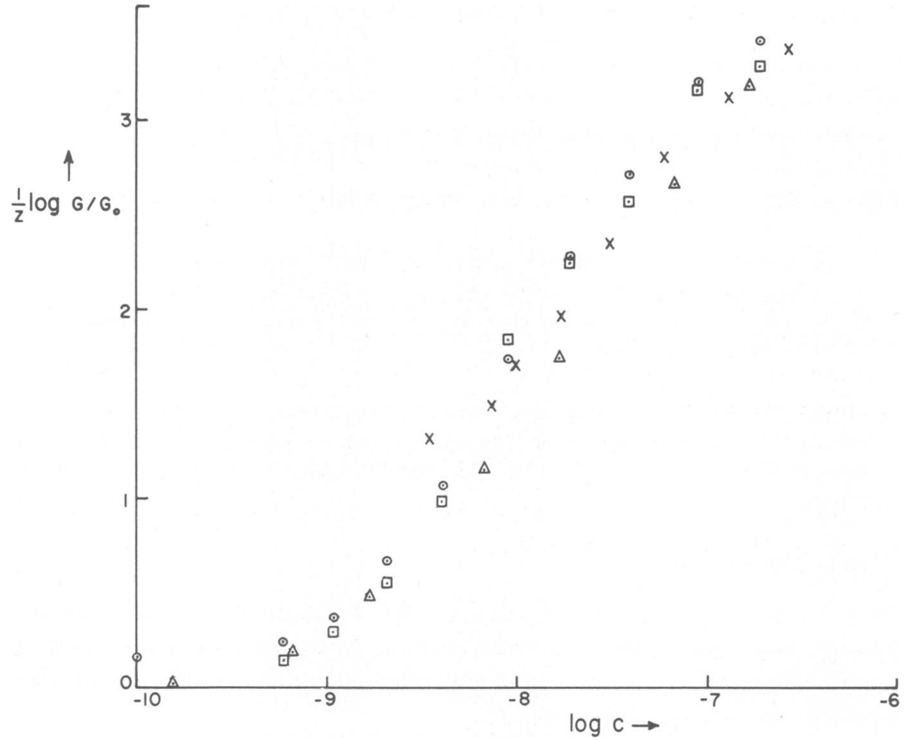

FIGURE 1 The change in the conductance G of a phosphatidyl ethanolamine membrane, induced by phloretin of aqueous concentration c (mol cm^{-3}), as reported by Andersen et al. (1976). The conductance in the absence of phloretin is denoted by G_0, and the valences of the monovalent membrane-permeant ions by z. The following ions were used: tetraphenylborate (o), carbonylcyanide-*m*-chlorophenylhydrazone (□), tetraphenylarsonium (X), and potassium in the presence of nonactin (Δ).

mon effect is involved that depends solely on the charge of the transported ion. Andersen et al. (1976) interpret this effect of phloretin as due to the adsorption of this dipolar molecule in the region of the existing membrane dipoles. These phloretin dipoles will orient themselves so as to reduce the existing membrane dipole potential, so that their adsorption will facilitate cation transport and hinder anion permeation.

The amount of phloretin adsorption can be inferred from its effect on anionic and cationic membrane conductance. With the data so obtained one can probe the mutual (dipole-dipole) interaction of adsorbed phloretin as a model for interactions among membrane-associated dipole molecules.

A direct measurement of phloretin adsorption has been reported by Jennings and Solomon (1976) for red blood cell membranes. Their data indicated Langmuir-type adsorption onto the red cell lipids, in addition to higher-affinity binding to nonlipid membrane constituents. Langmuir-type adsorption of the related compound phlorizin onto phospholipid vesicles had earlier been reported by Ehrenspeck (1975). Recently Cousin and Motais (1978) found that the inhibiting effect of phloretin analogues on chloride transport through red blood cell membranes correlates well with their membrane solubility and dipole moment.

THE MEASUREMENT OF PHLORETIN ADSORPTION

We will assume that the neutral dipolar molecules adsorbing onto the membrane are oriented parallel to each other. Let us denote with θ the angle between the direction of their dipole moment vectors and the membrane/water interface.

The introduction of such aligned dipoles will generate, in the direction normal to the membrane surface, a change, Δ, in the existing space-averaged dipole potential,

$$\Delta = (4\pi\mu N\Gamma \sin \theta)/\epsilon, \tag{1}$$

where μ is the dipole moment introduced by the adsorption of the molecule, Γ the surface excess in number of moles adsorbed per unit area, N Avogadro's number, and ϵ the effective dielectric constant in the plane of the adsorbing molecules. The resulting change in membrane conductance is then

$$G/G_0 = \exp[zF\Delta/RT], \tag{2}$$

where G and G_0 denote the conductance in the presence and absence of the dipolar adsorbate, respectively. When we rewrite Eq. 1 as

$$\Gamma = \epsilon\Delta/4\pi\mu N \sin \theta, \tag{3}$$

it is clear that Γ can be related directly to the experimentally observable ratio G/G_0 through Eq. 3, and

$$\Delta = (RT/zF) \ln (G/G_0). \tag{4}$$

THE ADSORPTION ISOTHERM

Isotherms for the adsorption of neutral molecules can, in general, be written (Delahay, 1965) as

$$\beta c = f(\Gamma) \tag{5}$$

$$\beta = \exp[-\Delta G_a/RT], \tag{6}$$

where ΔG_a denotes the free energy of adsorption, c the bulk concentration, and the function $F(\Gamma)$ describes the adsorption statistics. For Langmuir statistics, i.e., for adsorption onto a fixed number of equivalent sites, with Γ_m as the maximum value of Γ, we have

$$\beta c = \Gamma\Gamma_m/(\Gamma_m - \Gamma) = \{1/\Gamma - 1/\Gamma_m\}^{-1}. \tag{7}$$

In general, the free energy of adsorption contains a standard contribution, ΔG_a^0, as well as a Γ-dependent term representing adsorbate-adsorbate interactions and, consequently, $\beta = \beta_0\beta_i$ where β_0 only contains the standard term for adsorbate-adsorbens interactions, whereas β_i contains adsorbate-adsorbate interactions. In the absence of the latter, $\beta = \beta_0$ and Eq. 7 can be combined with Eq. 1 to form

$$1/\Delta = 1/\Delta_m + (\epsilon/4\pi\mu N\beta_0 \sin \theta) 1/c, \tag{8}$$

where the relation between Δ_m and Γ_m is like that between Δ and Γ (see Eq. 1). Eq. 8 pre-

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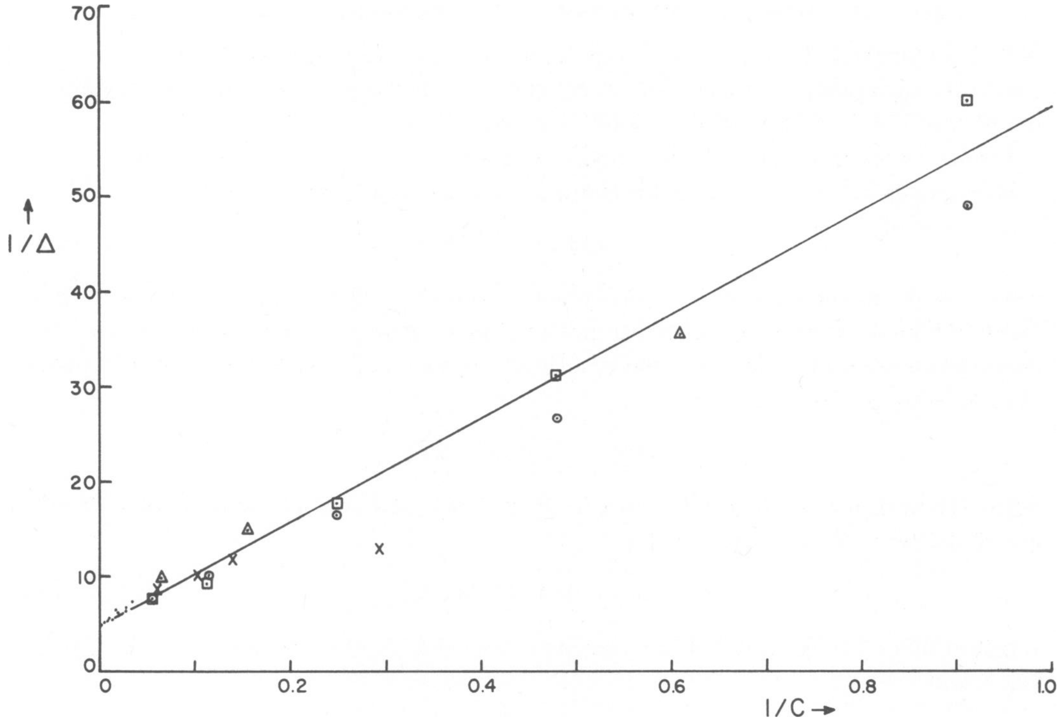

FIGURE 2 The experimental data of Fig. 1 plotted as $1/\Delta$ vs. $1/c$. The intercept as $1/c$ approaches zero yields $1/\Delta_m = 4.5\ \mathrm{V}^{-1}$ (hence $\Delta_m = 0.22$ V).

dicts a linear relation between $1/\Delta$ and $1/c$, and such a relation is indeed observed in Fig. 2 for the experimental data of Andersen et al. (1976). Clearly, a Langmuir isotherm fits the available experimental data quite well (but see also Fig. 3).

This result may appear surprising in view of the very strong influence exerted by adsorbed phloretin molecules on ion transport. Below we will show, however, that the dipole-dipole interactions between adsorbed phloretin molecules are much weaker than the interaction between phloretin dipoles and ions.

DIPOLE-DIPOLE INTERACTION

We first consider a single-point dipole, with dipole moment μ, and a point P at a distance r from that dipole. Both P and the dipole lie in the adsorption plane; we denote with θ the angle between the dipole moment vector and the adsorption plane, and with α the angle between the projection of the dipole moment vector onto the adsorption plane and the line connecting the dipole with point P. From simple electrostatics we then find for the resulting field F' at point P:

$$F' = (\mu/\epsilon r^3)(1 - 3\cos^2\theta\cos^2\alpha). \tag{9}$$

We now consider a "central" dipole surrounded by other dipoles. The field F, due to all surrounding dipoles, is considered to be distributed homogeneously with a dipole density of N molecules per unit area in the adsorption plane, excluding an area with radius $(\pi N\Gamma)^{-1/2}$

that represents the area occupied by the central dipole (Esin and Shikov, 1943; Grahame, 1958), and now follows as

$$F = (\mu N\Gamma/\epsilon) \int_{(\pi N\Gamma)^{-1/2}}^{\infty} (1/r^2) \int_0^{2\pi} (1 - 3\cos^2\theta\cos^2\alpha)\,d\alpha\,dr$$
$$= \pi^{3/2}\mu(2 - 3\cos^2\theta)(N\Gamma)^{3/2}/\epsilon. \qquad (10)$$

The corresponding energy per mole is

$$U_i = \tfrac{1}{2}N\mu F = \pi^{3/2}\mu^2 N^{5/2}(2 - 3\cos^2\theta)\Gamma^{3/2}/2\epsilon. \qquad (11)$$

Consequently,

$$\beta_i = \exp[-U_i/RT] = \exp[-\pi^{3/2}\mu^2 N^{5/2}(2 - 3\cos^2\theta)\Gamma^{3/2}/2\epsilon RT]$$
$$= \exp[-\mu^{1/2}\epsilon^{1/2}N(2 - 3\cos^2\theta)\Delta^{3/2}/16RT\sin^{3/2}\theta]. \qquad (12)$$

From Eq. 12 we can now estimate the order of magnitude of β_i. Let us assume that $\theta = 90°$, take $\epsilon \simeq 10^{-11}$ F cm^{-1} (i.e., a relative dielectric constant of 9) and the dipole moment of phloretin, $\mu = 5.6\ D = 2 \times 10^{-27}$ C cm. The ratio $(G/G_0)^{1/z}$ in the data set of Andersen et al. (1976) ranges from 1 to about 2×10^3, and hence Δ from 0 to 0.2 V, so that β_i ranges from 1 to about 0.7. Consequently, at a phloretin concentration that modifies ion conductance by a factor of 2,000, the mutual dipole-dipole interaction between adsorbed phloretin molecules modifies the value of the adsorption coefficient β by only about 30%.

We now make the customary Bragg-Williams approximation that, for weak interactions, the site statistics can be assumed to be unaffected. Substitution of Eq. 12 into Eq. 7 then leads to an isotherm which explicitly includes dipole-dipole interactions, and which can be written in terms of Δ and Δ_m as

$$\ln(c/\Delta - c/\Delta_m) = \ln(\epsilon/4\pi\mu N\beta_0\sin\theta)$$
$$+ \pi^{1/2}\epsilon^{1/2}N(2 - 3\cos^2\theta)\Delta^{3/2}/16RT\sin^{3/2}\theta. \qquad (13)$$

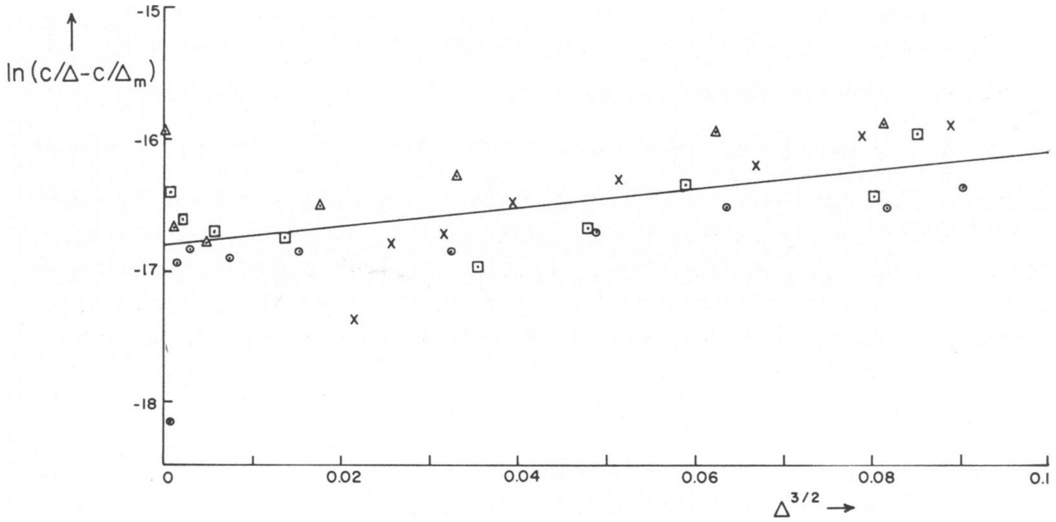

FIGURE 3 Plot of the data of Fig. 1 as $\ln(c/\Delta - c/\Delta_m)$ vs. $\Delta^{-3/2}$ for $\Delta_m = 0.22$ V; the line is drawn under slope 7 V$^{-3/2}$.

Fig. 3 shows a plot of $\ln(c/\Delta - c/\Delta_m)$ versus $\Delta^{3/2}$, which emphasizes the Γ-dependence of ΔG_a. Apparently such a dependence exists, and an improved fit is indeed obtained when dipole-dipole interactions are taken into account. The effect is small and of the predicted order of magnitude.

DISCUSSION

The observation that phloretin adsorption onto planar lipid bilayers can be represented quite well by a Langmuir isotherm agrees with the results of Jennings and Solomon (1976) on red blood cells. Both experimentally and theoretically, the dipole-dipole interaction is so much weaker than the ion-dipole interaction that the former has only a minor effect on the adsorption isotherm, even though the adsorbed phloretin can have an enormous effect on ion permeation through the membrane. Because the dipole-dipole interaction is so weak, the available data are insufficient to distinguish between the model presented here, and various alternatives, such as one including imaging effects, or one assuming a space-averaged, "smeared," potential profile.

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