5-1991

Domains and anomalous adsorption isotherms of dipalmitoylphosphatidylcholine membranes and lipophilic ions: pentachlorophenolate, tetraphenylborate, and dipicrylamine

Pavel Smejtek
Portland State University

S. Wang

Domains and anomalous adsorption isotherms of dipalmitoylphosphatidylcholine membranes and lipophilic ions: pentachlorophenolate, tetraphenylborate, and dipicrylamine

Pavel Smejtek and Shanru Wang

Department of Physics and Environmental Sciences and Resources Doctoral Program, Portland State University, Portland, Oregon 97207-0715 USA

ABSTRACT Dipalmitoylphosphatidylcholine (DPPC) vesicles acquire negative surface charge on adsorption of negatively charged pentachlorophenolate (PCP^-), and lipophilic ions tetraphenylborate ($TPhB^-$), and dipicrylamine (DPA^-). We have obtained (*a*) ζ-potential isotherms from the measurements of electrophoretic mobility of DPPC vesicles as a function of concentration of the adsorbing ions at different temperatures (25–42°C), and (*b*) studied the effect of PCP^- on gel-to-fluid phase transition by measuring the temperature dependence of ζ-potential at different PCP^- concentrations. The ζ-potential isotherms of PCP^- at 25, 32, and 34°C correspond to adsorption to membrane in its gel phase. At 42°C the ζ-potential isotherm corresponds to membrane in its fluid phase. These isotherms are well described by a Langmuir-Stern-Grahame adsorption model proposed by McLaughlin and Harary (1977. *Biochemistry.* 15:1941–1948). The ζ-potential isotherm at 37°C does not follow the single-phase adsorption model. We have also observed anomalous adsorption isotherms for lipophilic ions $TPhB^-$ and DPA^- at temperatures as low as 25°C. These isotherms demonstrate a gel-to-fluid phase transition driven by ion adsorption to DPPC membrane during which the membrane changes from weakly to a strongly adsorbing state. The anomalous isotherm of PCP^- and the temperature dependence of ζ-potential can be described by a two-phase model based on the combination of (*a*) Langmuir-Stern-Grahame model for each phase, (*b*) the coexistence of gel and fluid domains, and (*c*) depression of gel-to-fluid phase transition temperature by PCP^-. Within the anomalous region the magnitude of ζ-potential rapidly increases with increasing concentration of adsorbing species, which was characterized in terms of a Esin-Markov coefficient. This effect can be exploited in membrane-based devices. Comments are also made on the possible effect of PCP, as an uncoupler, in energy transducing membranes.

INTRODUCTION

It has been recognized that due to nonideal miscibility of lipids and other membrane components, the biological membrane can be regarded as an "archipelago" containing domains of different composition of lipids and proteins with different functions. The domain formation can be initiated by protons, multivalent ions, proteins, and other biologically active compounds (1, 2). It is to be expected that toxic amphiphilic molecules, such as chlorophenols, may be distributed unevenly between different membrane domains or may alter the segregation state of lipids.

We are interested in effects of chlorinated phenols on lipid bilayer membranes for several reasons: (*a*) chlorinated phenols, and among them pentachlorophenol (PCP), are highly toxic environmental pollutants (3, 4). These compounds belong to the class of lipophilic weak acids whose biological effect is the uncoupling of synthesis of ATP from electron transport (3, 5, 6). (*b*) The effect of weak acid uncouplers on membranes is complex (7). In addition to facilitation of transmembrane proton translocation (8, 9), the membranes become negatively charged due to the adsorption of ionized acid (7, 10); some are known to alter the dipolar potential of the membrane/water interface (11) and change the gel-to-fluid phase transition temperature of phosphatidylcholine membranes (12). (*c*) Upon adsorption to membranes, physicochemical properties of lipophilic uncouplers, such as the dissociation constant, are altered by the low polarity environment of the adsorption site (13). More detailed knowledge of adsorption of these compounds to lipid membranes and the associated changes in the physical and biochemical properties are a prerequisite for understanding the action of toxic compounds.

To understand the relationships between the adsorption characteristics, molecular structure of the membrane, and of adsorbing molecules, we have studied the adsorption of PCP^- to a simple, single component dipalmitoylphosphatidylcholine (DPPC) membrane using microelectrophoresis (14). It was shown that ζ-potentials of DPPC vesicles, in the presence of PCP^-, and lipophilic anions tetraphenylborate ($TPhB^-$), and dipicrylamine (DPA^-), above and below the gel-to-fluid transition temperature of DPPC membranes can be described by a combination of the Langmuir and Gouy-Chapman-Stern models. The properties of ζ-potential isotherms of DPPC membranes, in the presence of

Address correspondence to Dr. Pavel Smejtek.

0006-3495/91/05/1064/10 $2.00

PCP^-, $TPhB^-$, and DPA^-, were found to be consistent with a simple discrete charge model if the adsorption plane of the ions was 0.4–0.6 nm below the carbonyl oxygen layer of DPPC (14).

Here we describe ζ-potential isotherms that are anomalous in the sense that they cannot be described in terms of a simple, one component Langmuir model. These isotherms have been observed for PCP^- and lipophilic ions $TPhB^-$ and DPA^- within a limited range of temperatures and concentration of adsorbing species. We show that the anomalous behavior can be understood in terms of the PCP^- or lipophilic ion-induced isothermal transition of membrane from the gel into the fluid state and the coexistence of gel and fluid domains.

GLOSSARY

DPA	dipicrylamine
DPPC	dipalmitoylphosphatidylcholine
PCP	pentachlorophenol
TPhB	tetraphenylboron
$(A^-)_m$	membrane surface density of adsorbing ions A^-
$(A^-)_{mg}$, $(A^-)_{mf}$	membrane surface density of adsorbing ions A^- in the gel and fluid phase
$[A^-]_m$	concentration of adsorbing ions A^- at the aqueous side of membrane surface
$[A^-]_{tot}$	concentration of adsorbing ions in the vesicle suspension;
$[A^-]_0$	concentration of adsorbing ions in the aqueous portion of the vesicle suspension
C_{i0}	volume density of ions type i in the aqueous solution suspending vesicles
d	Debye characteristic distance equal to $(\Sigma_i \cdot z_i^2 e^2 C_{i0}/\epsilon\epsilon_0 kT)^{-1/2}$
e	unit of electric charge
ΔH^0	standard enthalpy change of lipids on gel-to-fluid transition
ΔH_{vH}	van't Hoff enthalpy
k	Boltzmann constant
K	association constant of adsorbing species A^-
K_g, K_f	association constant of adsorbing species A^- with the membrane in the gel and fluid phase, respectively
$[L]$	concentration of lipids in the suspension of vesicles
N_{AV}	Avogadro's number
P_l	membrane surface area of lipid molecule
P_{lg}, P_{lf}	membrane surface area of lipid in gel and fluid phase
P_s	membrane surface area of ion adsoption site
P_{sg}, P_{sf}	membrane surface area of ion adsoption site in gel and fluid membrane phase
R	gas constant
s	distance between the membrane surface and the surface of shear
ΔS^0	standard entropy change of lipids on gel-to-fluid transition
T	absolute temperature
T_0	gel-to-fluid transition temperature of membrane in the absence of adsorbing ions A^-
T_{0s}	gel-to-fluid transition temperature of membrane in the presence of adsorbing ions A^-
ΔT	shift to gel-to-fluid transition temperature
U_g, U_f	contribution to chemical potential of lipids due to the presence of adsorbed A^-
V_m	electric potential at the membrane surface
X_{gl}, X_{fl}	mole fraction of lipids in the gel and fluid phase
z_i	valency of ion i in the suspending aqueous solution
α	fluid fraction of membrane lipid equal to the degree of conversion from the gel into the fluid phase
μ	electrophoretic mobility
ϵ	relative dielectric constant of aqueous medium
ϵ_0	permittivity of free space
η	viscosity of aqueous medium
ζ	zeta potential, electric potential at the shear surface i.e., at the surface where the aqueous medium flows with respect to the surface of lipid vesicle
σ_m	membrane surface density of electric charge
μ_{gl}^0, μ_{fl}^0	standard chemical potential of lipids in the gel and fluid phase

MATERIALS, PROCEDURES, AND METHODS

Materials

Dipalmitoylphosphatidylcholine was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL), pentachlorophenol (99% pure), sodium tetraphenylborate, and dipicrylamine from Aldrich Chemical Co. (Milwaukee, WI).

Procedures

Multilamellar DPPC vesicles were prepared by (*a*) depositing a thin lipid layer in round bottom flask by evaporation from a chloroform solution of the lipid, (*b*) adding suspending medium at ~45°C (which is above the gel-to-fluid phase transition temperature of DPPC) and (*c*) manually shaking the flask containing the suspending medium. The suspending medium used in the electrophoretic mobility studies contained 0.03 M KCl and a potassium phosphate/citrate/borate buffer (0.002/0.002/0.0005 M) at pH 10. High pH values are necessary in order to exclude adsorption of neutral PCP. More details are given in reference 12.[1]

A Mark-1, an electrophoretic mobility instrument from Rank

[1]Freshly prepared vesicle suspensions were used. On the time scale of experiments we have not noticed any time-dependent changes of electrophoretic mobility indicating that degradative processes were absent.

Instruments (Bottisham, Cambridge, England) with a thermally insulated water tank, was used for the determination of ζ-potential of DPPC vesicles. Each ζ-potential data point was obtained from ~25 vesicles for two polarities of the applied drift electric field. The velocity of vesicles was measured in the vicinity of the stationary layer as a function of their radial position in the electrophoretic cell. The drift velocity at the stationary layer was obtained using linear regression. The Helmholtz equation, $\mu = \epsilon\epsilon_0\zeta/\eta$ (15), where ϵ is the dielectric constant of water, and η the viscosity of water at a given temperature, was used to convert electrophoretic mobility data into ζ-potential.

Methods: one-phase adsorption model

Adsorption of ions to membranes can be described by a model combining Langmuir adsorption isotherm with the Guoy-Chapman theory of space charge regions adjacent to a charged membrane. We have adopted a version of a model proposed in the earlier work of McLaughlin and Harary (16).

Below we outline a one-phase model capable of reproducing the ζ-potential isotherms of the gel and the fluid DPPC membranes (14).

The major elements of the one-phase adsorption model are (*a*) the Langmuir adsorption isotherm,

$$(A^-)_m = K[A^-]_m [1/P_s - (A^-)_m], \quad (1)$$

where we designate $(A^-)_m$ as the membrane surface density and $[A^-]_m$ as the interfacial aqueous concentrations of the adsorbing species, K is the association constant, and P_s is the membrane surface area of an ion "adsorption site."

(*b*) The condition of electroneutrality, stating that the surface charge of membrane is compensated by the space charge of opposite polarity within the diffuse double layer, is

$$\sigma_m + [2kT\epsilon\epsilon_0 \Sigma C_{i0}[\exp(-z_i eV_m/kT) - 1]]^{1/2} = 0. \quad (2)$$

For uncharged membranes, such as DPPC, the surface charge density of the membrane is determined solely by the adsorbed PCP^- or lipophilic ions,

$$\sigma_m = -e(A^-)_m. \quad (3)$$

The ζ-potential is obtained from the surface potential V_m, the shear plane distance s, and Debye screening distance d,

$$\zeta = V_m \exp(-s/d). \quad (4)$$

The Debye screening distance was obtained from the salt and buffer concentrations and the distance of the shear plane, $s = 0.255$ nm, was determined earlier (14).

(*c*) The reduction of the interfacial concentration of the adsorbing species due to the electrostatic repulsion between the membrane charge and the negatively charged adsorbing species within the aqueous interfacial region is

$$[A^-]_m/[A^-]_0 = \exp(eV_m/kT). \quad (5)$$

$[A^-]_0$ is the bulk aqueous concentration of the adsorbing ions and V_m is the membrane surface potential.

(*d*) Redistribution of A^- between the aqueous medium and the membrane surface is accounted for by

$$[A^-]_{tot} = [A^-]_0 + (A^-)_m [L] N_{AV} P_l. \quad (6)$$

The above model applies individually to both the gel and fluid states of the membrane. The ability of the model to fit the ζ-potential isotherms of PCP-modified membranes is demonstrated by the broken curves in Fig. 1, *a–d*.

There are two adsorption parameters to characterize adsorption to each membrane phase: the association constant, K, and the membrane surface area of the "adsorption site," P_s. In the present study we use the adsorption parameters determined in previous work (14). These are summarized in Table 1 and are used in conjunction with the following values for the membrane surface areas of lipids: $P_{ls} = 0.46$ nm^2 (17) and $P_{lf} = 0.71$ nm^2 (18). The curves depicting ζ-potential isotherms of individual gel and fluid states of DPPC membranes in the presence of PCP^-, $TPhB^-$, and DPA^- are shown in Figs. 1–4 and were obtained from the above model.

EXPERIMENTAL RESULTS

ζ-potential isotherms

Pentachlorophenol

The dependence of the ζ-potential of DPPC vesicles on PCP^- concentration at various temperatures is shown in Figs. 1 and 2. We show that for temperatures 25, 32, and 34°C (Fig. 1, *a–c*) the ζ-potential isotherms are identical. Because the transition temperature of DPPC is ~41°C, these isotherms characterize adsorption of PCP^- to phosphatidylcholine membrane containing lipids in the ordered, gel state. Above the gel-to-fluid phase transition the adsorption of PCP^- is greater, the ζ-potentials obtained at 42°C (Fig. 1 *d*) are more negative compared to those at lower temperatures. The broken curves illustrate that the ζ-potential isotherms of DPPC membranes in both the gel and fluid states can be well-described in terms of combined Langmuir-Stern-Grahame model.

In Fig. 2 we present the dependence of ζ-potential on PCP concentration obtained at 37°C. The upper broken curve depicts the ζ-potential isotherm for the membrane in the gel state, and the lower one for the fluid state as obtained from the one-phase Langmuir-type model. The experimental isotherm is anomalous, at low PCP concentration the data coincide with the isotherm for the gel state and at higher PCP concentrations the data progressively deviate from the gel isotherm and finally merge with the fluid state isotherm. Clearly, the anomalous ζ-potential isotherm is associated with the PCP-driven isothermal transition of the membrane from the low adsorbing gel state into the strong adsorbing fluid state.

Tetraphenylborate and dipicrylamine

Lipophilic ions $TPhB^-$ and DPA^- exhibit similar effects. $TPhB^-$ and DPA^- also perturb the gel-to-fluid phase transition, as anomalous ζ-potential isotherms were

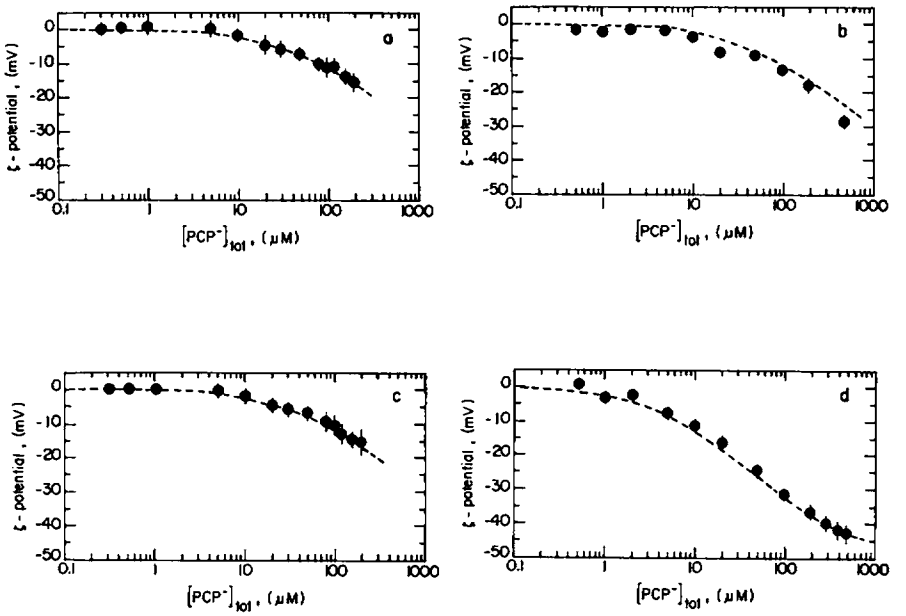

FIGURE 1 ζ-potential isotherms of DPPC multilayered vesicles in the presence of PCP^-. Conditions: 0.03 M KCl, phosphate/citrate/borate buffer 0.002/0.002/0.0005 M, pH 10. Isotherms obtained at 25°C (*a*), 32°C (*b*), and 34°C (*c*) correspond to the gel state membrane whereas at 42°C (*d*) to the fluid state of a DPPC membrane. These are called normal isotherms because they can be understood in terms of a simple Langmuir adsorption model. The broken curves illustrate the model predictions for adsorption parameters given in the text.

found at temperatures as low as 25°C (Figs. 3 and 4).[2] The upper and lower curves indicate the ζ-potential isotherms for the gel and fluid state of DPPC membranes determined earlier (14). The major differences between the properties of the lipophilic ions and PCP^- that can be inferred from Figs. 2–4 are (*a*) that the association constants of $TPhB^-$ and DPA^- for DPPC membranes are more than 10 times greater than for PCP^- as follows from the shifts of the ζ-potential isotherms along the concentration axis, and (*b*) there is a smaller difference, compared to PCP^-, in the adsorption of $TPhB^-$ and DPA^- to the gel and fluid state of the membrane. The existence of the anomalous isotherms for $TPhB^-$ and DPA^- at 25°C, in contrast to the absence of anomalous behavior for PCP^- up to 34–37°C, is in large part due to the larger size of these lipophilic ions and thus to a greater degree of disorder induced in the membrane by their presence.

TABLE 1 Adsorption parameters of PCP^-, $TPhB^-$, DPA^-, and DPPC membranes used in theoretical models, from reference 14.

Ion	K_g	K_f	P_{sg}	P_{sf}
	M^{-1}	M^{-1}	nm^2	nm^2
PCP^-	4.9×10^3	4.5×10^4	5.4	4.5
$TPhB^-$	3.1×10^5	5.9×10^5	5.0	4.1
DPA^-	2.5×10^5	7.4×10^5	5.9	5.2

[2]The DPA^- concentration range was limited because concentrated DPA^- solutions were strongly colored and it was difficult to see the vesicles.

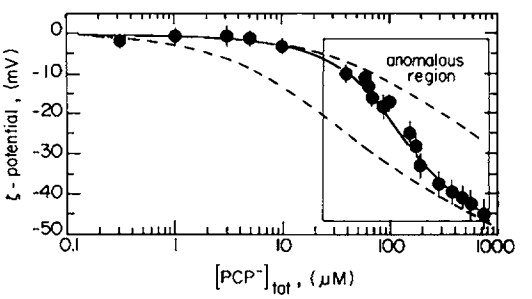

FIGURE 2 Anomalous ζ-potential isotherm of PCP^- and DPPC multilayered vesicles observed at 37°C. Experimental conditions were identical to those given in Fig. 1. The broken curves indicate the isotherms for the gel state (*upper*) and the fluid state (*lower*) of DPPC membrane. The solid curve shows the theoretical isotherm based on the model described in the text, with adsorption characteristics given in Table 1 and van't Hoff enthalpy, ΔH_{vH} = 7,500 kcal/mol.

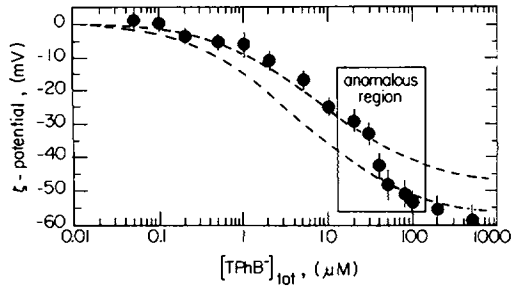

FIGURE 3 Anomalous ζ-potential isotherms of tetraphenylborate and DPPC multilayered vesicles observed at 25°C. Experimental conditions were the same as in Fig. 1. The broken curves indicate the isotherms for the gel state (*upper*) and the fluid state (*lower*) of DPPC membrane.

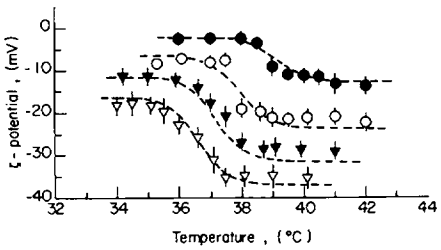

FIGURE 5 Dependence of ζ-potential of DPPC multilayered vesicles on temperature in the presence of PCP^-. Conditions: 0.03 M KCl, phosphate/citrate/borate buffer 0.002/0.002/0.0005 M, pH 10. PCP^- concentrations (top down): 10 μM, 40 μM, 100 μM, 200 μM. The broken curves show the theoretical temperature dependence based on the model described in the text with adsorption characteristics given in Table 1 and van't Hoff enthalpy, ΔH_{vH} = 7,500 kcal/mol.

Temperature dependence of ζ-potential in the presence of PCP^-

In Figs. 1–4 the ζ-potential was obtained at a constant temperature as a function of increasing concentration of adsorbing ions. In Fig. 5 we plot the temperature dependence of ζ-potential at a constant concentration of PCP^- from a related experiment illustrating the effect of gel-to-fluid transition of membrane on its ζ-potential. The ζ-potential undergoes a rather abrupt increase in the magnitude as the temperature of the vesicle suspension is increased. This change is again due to the transformation of the membrane from the gel into the fluid state. Another notable feature of the data is that the midpoint transition temperature decreases with the increasing concentration of PCP^-. The curves depict the theoretical results computed from the model described below.

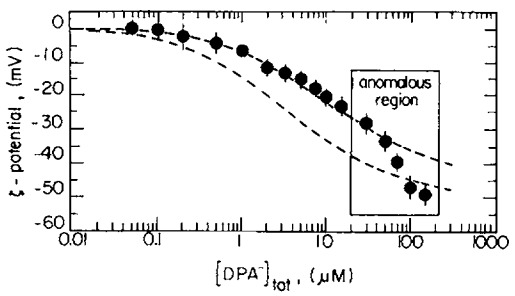

FIGURE 4 Anomalous ζ-potential isotherms of dipicrylamine and DPPC multilayered vesicles observed at 25°C. Experimental conditions were the same as in Fig. 1. The broken curves indicate the isotherms for the gel state (*upper*) and the fluid state (*lower*) of DPPC membrane.

THEORY AND DISCUSSION

The anomalous ζ-potential isotherms depicted in Figs. 2–4 demonstrate the ion-induced isothermal transition of DPPC membrane from the gel into the fluid state. At low concentrations of the adsorbing ions, PCP^-, $TPhB^-$, and DPA^-, the anomalous isotherms initially after that the gel phase isotherms and then transforms into the fluid phase isotherms. Because the largest difference between the ζ-potential isotherms for the gel and fluid state has been observed for PCP and because PCP is the compound of major interest as an uncoupler, the objective is to develop a model for the anomalous ζ-potential isotherm. The model can be extended to $TPhB^-$ and DPA^-.

Note that the anomalous ζ-potential isotherms were obtained from electrophoretic mobility measurements not involving any temperature scans. For each data point in Figs. 2–4 the electrophoretic cell was filled with a different solution, preequilibrated at the given temperature for many hours. We therefore assume that within the gel-to-fluid transition region of the anomalous isotherms there is an equilibrium distribution of gel and fluid domains. The domains are schematically depicted in Fig. 6. Each type of domain has its own specific adsorption characteristics listed in Table 1.

We show that the anomalous adsorption isotherms can be understood in terms of: (*a*) a two-phase Langmuir-Stern-Grahame model, (*b*) shifts of the gel-to-fluid phase transition temperature induced by the adsorbed ions, and (*c*) the coexistence of laterally segregated fluid and gel domains with adsorption characteristics of gel and fluid membrane phases.

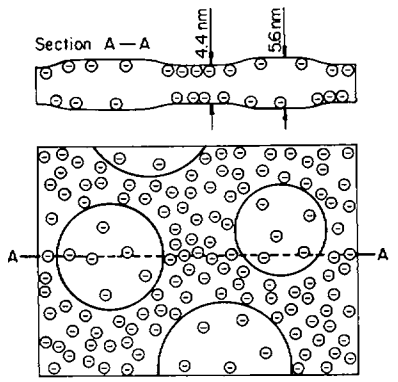

FIGURE 6 Schematic diagram of membrane surface depicting gel domains in the fluid DPPC membrane. The fluid regions of the DPPC membrane are considerably thinner due to disordered hydrocarbon chains and have a greater charge density due to adsorbed PCP^- or lipophilic ions.

Two-phase adsorption model

The adsorption model proposed for the anomalous isotherms uses two one-phase adsorption models (outlined in Methods, Eqs. 1–6), to describe adsorption to the gel and fluid membrane phases. Subsequently the quantities related to the gel and fluid phase are designated by subscripts "g" and "f."

Due to the different adsorption characteristics of the gel and fluid phases of DPPC membrane (see Table 1), the gel and the fluid regions have different surface charge densities, different surface potential, V_{mg} and V_{mf}, and consequently different interfacial concentrations $[A^-]_{mg}$ and $[A^-]_{mf}$. This distinction between the gel and the fluid domains is accounted for by solving the one-phase adsorption models individually for the gel and fluid phases (Eqs. 1–5) for a common value of the bulk aqueous concentration $[A^-]_0$. The distribution of the adsorbing ions between the gel and fluid membrane regions and the aqueous solution is accounted for by the following balance equation,

$$[A^-]_{tot} = [A^-]_0 + ([A^-]_{mf}\alpha P_{lf} + [A^-]_{mg}(1 - \alpha)P_{lg})[L]N_{AV}, \quad (7)$$

α represents the degree of conversion of the membrane from the gel into the fluid state. In the two-phase model Eq. 7 replaces Eq. 6.

The ζ-potential of the two-phase vesicles is determined from the average density of the surface charge σ_m. The contributions of the surface charge density of the gel and fluid regions are weighted according to the degree of conversion from the gel into the fluid state, α,

$$\sigma_m = \alpha\sigma_{mf} + (1 - \alpha)\sigma_{mg}. \quad (8)$$

At the present time it is not known how large the gel and the fluid domains are. Because the bilayer thicknesses vary considerably from 4.4 nm (19) for the fluid domains to 5.6 nm (19, 20) for the gel regions, and each type of the domain is assumed to have a different surface charge density, it is not clear as to what electrostatic potential determines the interfacial concentration $[A^-]_m$ (Eq. 5). It is expected that the solution of Poisson's equation for the heterogenous surface would predict well defined and different membrane surface potentials, V_{mg} and V_{mf}, for each type of domain if the domain size is larger or comparable to the Debye screening distance. For small domains the electrostatic potential is not expected to vary significantly from some average value. In view of this uncertainty, we considered two subclasses of the model.

In the subclass of large domains we assume that the interfacial concentrations $[A^-]_{mg}$ and $[A^-]_{mf}$ are different and defined by two equations (Eq. 5) in response to two different values of membrane surface potentials, V_{mg} and V_{mf}. In the subclass of small domains we assume $[A^-]_{mg} = [A^-]_{mf}$ due to the average surface potential $V_m = V_{mg} = V_{mf}$ determined by the average surface charge density.

Shift of the gel-to-fluid phase transition temperature

At the gel-to-fluid phase transition the chemical potential of lipids in both phases are equal,

$$\mu^0_{gl} + RT \ln X_{gl} + U_g = \mu^0_{fl} + RT \ln X_{fl} + U_f. \quad (9)$$

The quantities U_g and U_f are the additional contributions to the chemical potential of lipids representing the changes due to the adsorbed species. The unitary free energy change of the gel-to-fluid conversion is equal to

$$\mu^0_{fl} - \mu^0_{gl} = \Delta H^0 - T\Delta S^0 = RT \ln (X_{gl}/X_{fl}) - (U_f - U_g). \quad (10)$$

The shift of the gel-to-fluid phase transition temperature follows from Eq. 10 and from the assumption that ΔH^0 and ΔS^0 are not affected by the presence of A^-,

$$\Delta T = T_{0s} - T_0 = \left(\frac{RT_{0s}T_0}{\Delta H^0}\right) \ln (X_{fl}/X_{gl}) + \frac{(U_f - U_g)T_0}{\Delta H^0}. \quad (11)$$

The mole fractions of lipids X_{fl} and X_{gl} are dependent on the degree of adsorption of A^- to the membrane in each phase,

$$X_{gl} = \frac{1/P_{lg}}{1/P_{lg} + (A_m)_g} \qquad X_{fl} = \frac{1/P_{lf}}{1/P_{lf} + (A_m)_f}. \quad (12)$$

The mole fractions of lipids in the gel and fluid domains will be different due to the different adsorption proper-

ties of the gel and fluid domains. These are obtained from the adsorption model described above.

Two models for the PCP-induced change of the gel-to-fluid phase transition temperature were considered. Model-1 was based on a simple ideal solution theory in which $U_f = U_g = 0$ and the change of the transition temperature was solely determined by the changes in the mole fractions of the lipids due to the PCP^- present in the membrane. In model 2 we consider $U_f \neq U_g \neq 0$.

For model 1 we obtain

$$\Delta T = \frac{RT_{0s}T_0}{\Delta H^0} \ln (X_{fl}/X_{gl}). \quad (13)$$

It follows that as a result of greater degree of adsorption of PCP^- to the fluid phase rather than to the gel phase (see Fig. 1, *a–d*), the ratio X_{fl}/X_{gl} is less than one. Consequently ΔT is negative, in agreement with the experimental results (see Fig. 5).

The changes in phase transition temperature determined from the ζ-potential studies are shown in Fig. 7. It turned out that the shifts of the transition temperature predicted from Eq. 13 and the adsorption model, for adsorption parameters fitting the ζ-potential isotherms in Fig. 1, *a–d*, are (results not shown) only in qualitative agreement with the experimental results. The ideal solution theory model (model 1) based on Eq. 13 underestimates the experimental data. Studies of membrane perturbation by optical membrane probes (21, 22), calcium channel blocking drugs (23), and antitumor drugs (24) indicate that the applicability of the ideal solution theory may strongly depend on the molecular structure of the membrane perturbing species. To make a conclusive judgement on the applicability of model 1, i.e., Eq. 13, to the observed changes of phase transition temperature induced by PCP^-, one would also need to validate the assumption of the independence of the

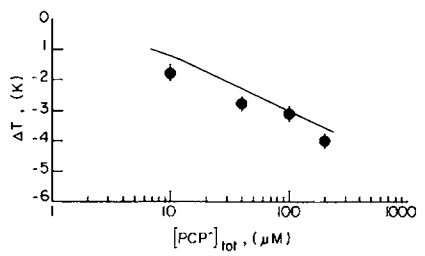

FIGURE 7 Shift of the gel-to-fluid transition temperature in DPPC membranes as a function of concentration of PCP^- in the vesicle suspension. The experimental data points were obtained from the temperature dependence of the ζ-potential at a given concentration of PCP in the suspension, $[PCP^-]_{tot}$. The solid curve illustrates the prediction of model 2 described in the text.

transition enthalpy, ΔH^0, on PCP^- concentration. Studies with oxonol dyes as probes of charge separation in membranes (21) and calcium channel blockers, specifically diltiazem and verapamil (23), indicated that the calorimetric enthalpy of the gel-to-fluid transition of DMPC significantly decreased in their presence at the level of several mole percent.

In our model we used $\Delta H^0 = 8.8$ kcal/mol (26). The fit of Eq. 13 to the experimental results would require that ΔH^0 decreases with the increasing concentration of PCP^-, which itself would contradict the assumption that PCP^- and DPPC form ideal solutions. To our knowledge there are no ΔH^0 data available for PCP^--modified DPPC membranes; a detailed study of the thermotropic behavior of phospholipids modified by PCP^- by high-sensitivity differential scanning calorimetry is highly desirable.

We have found an acceptable fit (shown in Fig. 7) to the dependence of ΔT on PCP^- concentration assuming that $U_f = 0$ and that the free energy of the gel phase increases in proportion to the mole fraction of adsorbed PCP^-, viz.

$$U_g = RT\left[\frac{(A_m^-)_g}{1/P_{lg} + (A_m^-)_g}\right]. \quad (14)$$

The nonideal solution model (model 2), i.e., the combination of the adsorption model used for each phase to determine the mole fractions X_{gl} and X_{fl} (Eqs. 12) with U_g given by Eq. 14, and ΔT by Eq. 11 is further used to predict the value of the gel-to-fluid phase transition temperature T_{0s}.[3]

Fluid fraction and transition width

The width of the ζ-potential transition associated with the conversion of the membrane from the gel into the fluid state in the presence of PCP was taken into account by using van't Hoff enthalpy, H_{vH} (25, 26). The degree of conversion α, at a given temperature, T, is given by

$$\frac{1}{T} - \frac{1}{T_{0s}} = \frac{R}{\Delta H_{vH}} \ln\left(\frac{1-\alpha}{\alpha}\right). \quad (15)$$

The fluid fraction α obtained from Eq. 15 is consequently used in the adsorption model from which the theoretical value of ζ-potential is determined.

We found rather small differences in the predicted ζ-potential results (comparable to experimental errors) between the ζ-potential determined from the large and

[3]Eq. 14 should be regarded only as an empirical description of the increase in the chemical potential of lipids in the gel state in the presence of PCP^-. One may speculate that this is due to distortions caused by the introduction of PCP^-.

small domain approximation mentioned earlier. Subsequently, all the computed results are based on the large domain approximation and van't Hoff enthalpy, ΔH_{vH} = 7,500 kcal/mol.

Overall, the theoretical ζ-potential results are in good agreement with the experimental data: (*a*) the model successfully reproduces the transition of the ζ-potential isotherm from the gel phase isotherm at low PCP^- concentration into the fluid isotherm at high PCP^- concentrations, the anomalous ζ-potential isotherm of PCP^- (Fig. 2, *solid curve*), and (*b*) the temperature dependence of ζ-potential for different PCP^- concentrations (Fig. 5, *broken curves*).

Esin-Markov coefficient of the transition region

Within the central portion of the anomalous adsorption isotherm the magnitude of the ζ-potential changes rapidly with the concentration of PCP^- as the membrane converts from the weakly adsorbing gel phase into the highly adsorbing fluid phase. The steepness of the membrane potential change with the concentration of adsorbing ions can be measured in terms of the Esin-Markov coefficient, $dV/d(\log [A^-]_{tot})$, which for the nerstian distribution has a value of 2.3 *kT/e*. The Esin-Markov coefficient has been used to measure the effects due to the discreteness of charge (27). In the present case it is used to measure the rapid change of the ζ-potential, or the membrane surface potential, caused not by the discrete charge effect but due to the rapid change of membrane adsorption of ions on the transition from the gel to the fluid phase. In the present case the value of the Esin-Markov coefficient depends upon: (*a*) the difference of ζ-potential isotherms in the fluid and gel states and (*b*) the width of the ζ-potential transition, and thus upon the value of van't Hoff enthalpy. In Fig. 8 we plot the relationship between the Esin-Markov coefficient and van't Hoff enthalpy for PCP^- and DPPC membrane determined from the numerical solution of the above model. The value of the normalized Esin-Markov coefficient, obtained from the anomalous region of the isotherm, is ~0.75, i.e., subnernstian.

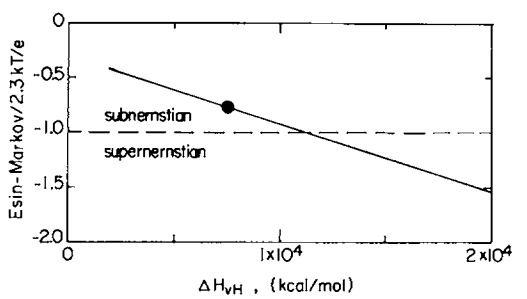

FIGURE 8 Dependence of normalized Esin-Markov coefficient, $(\partial V_m / \partial \log [A^-]_{tot})/(2.3\,kT/e)$, on van't Hoff enthalpy, ΔH_{vH} of the ζ-potential transition. The Esin-Markov coefficient was computed for the central portion of the anomalous ζ-potential isotherm using the adsorption characteristics of PCP^- given in Table 1.

The high values of Esin-Markov coefficient within the transition region of the anomalous isotherms are of interest for the design of molecular devices and sensors employing the gel-to-fluid phase transition of supported lipid membranes.

Comment on lipophilic ions

It turned out that the nonideal solution model (model 2) that satisfactorily predicted the anomalous ζ-potential isotherm of DPPC membranes in the presence of PCP^- failed to reproduce the isotherms of lipophilic ions $TPhB^-$ and DPA^-. The reason is that the shifts of the gel-to-fluid transition temperature induced by $TPhB^-$ and DPA^- are much greater than those induced by PCP^-. Model 2 does not predict a ΔT as large as 16°C required to reproduce the anomalous ζ-potential region observed for $TPhB^-$ and DPA^- at 25°C. $TPhB^-$ and DPA^- have similar adsorption characteristics (Table 1) and exhibit, due to their larger size, larger structural disturbances within the phospholipid bilayer, as compared to those for PCP^-. The model proposed here for the anomalous ζ-potential isotherm of PCP^- can be extended to $TPhB^-$ and DPA^- providing that data on ΔT dependence on lipophilic ion concentration are available.

There is active interest in understanding the formation and properties of membrane domains because it is likely that domains determine the topology of biological membranes and that enzymatic activity of membrane proteins is regulated by the physical properties of lipid domains. Membrane studies using direct visualization of membrane domains (28, 29), fluorescence recovery methods (30, 31), quick freeze techniques coupled with differential scanning calorimetry (32), and scanning tunneling microscopy (33) provide information on the existence and properties of domains in model lipid membranes and biomembranes. The anomalous ζ-potential isotherms found for PCP^-, $TPhB^-$, and DPA^- can be understood in terms of the coexistence of domains with different adsorption properties.[4]

[4]It was pointed out by one of the reviewers that if the gel phase is rippled, it is expected to exhibit both the solid and fluid characteristics. The fluid regions are contained in the apices, and the solid regions along the edges of ripples. We expect that due to the greater affinity of the adsorbing ions for the disordered regions of the membrane, the fluid domains originate and grow from the apical regions of the rippled bilayer.

This work directly demonstrates isothermally driven gel-to-fluid phase transition of lipids by an uncoupler and environmental pollutant, PCP. The anomalous ζ-potential and consequently the anomalous adsorption isotherms of PCP^- and DPPC membranes can be understood in terms of a two-phase adsorption model based on the following: Langmuir-Stern-Grahame adsorption isotherms for each phase, PCP-induced shifts of the phase transition temperature, and the coexistence of gel and fluid domains with adsorption properties characteristic specific for each phase. The results presented here provide additional insight into action of PCP in the lipid matrix of mitochondrial membranes: (*a*) PCP^- is expected to adsorb preferably into the disordered regions of the bilayer with a low density of negative charge, (*b*) due to this preferential adsorption it is expected that protonophoretic and therefore uncoupling activity of PCP will predominate in the less ordered regions of the lipid matrix. These regions will act as local sinks for protons generated by the proton pumps. The domains of disordered lipids, due to the adsorbed PCP^-, will also acquire greater negative charge and create localized patches with higher proton concentration at the membrane surface. (*c*) PCP^- decreases the free energy of disordered lipids relative to that of the ordered lipids. It is therefore expected that PCP^- contributes to stabilization of disordered regions in the biomembranes and in the lateral redistribution of membrane components.

We thank Dalibor Smejtek for technical assistance.

The research was supported by National Institutes of Health grant ES-05202. This paper is the Environmental Sciences and Resources Program publication No. 265.

Received for publication 1 October 1990 and in final form 17 January 1991.

REFERENCES

1. Cevc, G., and D. Marsh. 1987. Phospholipid Bilayers. Physical Principles and Models. ch. 13. John Wiley & Sons, New York.
2. Gennis, R. B. 1989. Biomembranes. Molecular Structure and Function. ch. 4. Springer-Verlag New York Inc., New York.
3. Ahlborg, U. G., and T. M. Thurnberg. 1980. Chlorinated phenols. Occurrence, toxicity, metabolism, and environmental impact. *CRC Crit. Rev. Toxicol.* 7:1–35.
4. Renner, G., C. Hopfer, and J. M. Gokel. 1986. Acute toxicities of pentachlorophenol, pentachloroanisole, tetrachlorohydroquinone, tetrachlorocatechol, tetrachlororesorcinol, tetrachlorodimethoxybenzenes and tetrachlorobenzenediol diacetates administered to mice. *Toxicol. Environ. Chem.* 11:37–50.
5. Weinbach, E. C., and J. Garbus. 1965. The interaction of uncoupling phenols with mitochondria and mitochondrial protein. *J. Biol. Chem.* 240:1811–1819.
6. Bakker, E. P., E. J. Van den Heuvel, A. H. C. A. Wiechmann, and K. Van Dam. 1973. A comparison between the effectiveness of uncouplers of oxidative phosphorylation in mitochondria and in different artificial membrane system. *Biochim. Biophys. Acta.* 292:78–87.
7. McLaughlin, S. G. A., and J. P. Dilger. 1980. Transport of protons across membranes by weak acids. *Physiol. Rev.* 60:825–863.
8. Slater, E. C. 1987. Review: the mechanism of conservation of energy of biological oxidations. *Eur. J. Biochem.* 166:489–504.
9. Smejtek, P., K. Hsu, and W. H. Perman. 1976. Electrical conductivity of lipid bilayer membranes induced by pentachlorophenol. *Biophys. J.* 16:319–336.
10. Smejtek, P., S. Wang, and A. W. Barstad. 1987. Adsorption of ionized and neutral pentachlorophenol to phosphatidylcholine membranes. *Biochim. Biophys. Acta.* 905:213–221.
11. Reyes, J., and D. J. Benos. 1984. Changes in interfacial potentials induced by carbonylcyanide phenylhydrazone uncouplers: possible role in inhibition of mitochondrial oxygen consumption and other transport processes. *Membr. Biochem.* 5:243–268.
12. Smejtek, P., A. W. Barstad, and S. Wang. 1989. Pentachlorophenol-induced change of zeta-potential and gel-to-fluid transition temperature in model lecithin membranes. *Chem. Biol. Interact.* 71:37–61.
13. Smejtek, P., A. W. Barstad, and K. Hsu. 1987. Dielectric properties of adsorption/ionization site of pentachlorophenol in lipid membranes. *Biochim. Biophys. Acta.* 902:109–127.
14. Smejtek, P., and S. Wang. 1990. Adsorption to DPPC membranes in gel and fluid state: pentachlorophenolate, dipicrylamine and tetraphenylborate. *Biophys. J.* 58:1285–1294.
15. Aveyard, R., and D. A. Haydon. 1973. An Introduction to the Principles of Surface Chemistry. Cambridge University Press, London. 52–57.
16. McLaughlin, S., and H. Harary. 1976. The hydrophobic adsorption of charged molecules to bilayer membranes: a test of applicability of the Stern equation. *Biochemistry.* 15:1941–1948.
17. Wiener, M. C., R. M. Suter, and J. F. Nagle. 1989. Structure of the fully hydrated gel phase of dipalmitoylphosphatidylcholine. *Biophys. J.* 55:315–325.
18. Lis, L. J., M. McAlister, N. Fuller, R. P. Rand, and V. A. Parsegian. 1982. Interactions between neutral phospholipid bilayer membranes. *Biophys. J.* 37:657–666.
19. Seelig, J., and A. Seelig. 1980. Lipid conformation in model membranes and biological membranes. *Q. Rev. Biophys.* 13:19–61.
20. Wiener, M. C., R. M. Suter, and J. F. Nagle. 1989. Structure of the fully hydrated gel phase of dipalmitoylphosphatidylcholine. *Biophys. J.* 55:315–325.
21. Fumero, J., B. P. Bammel, H. Hopkins, and J. C. Smith. 1988. The effect of potential-sensitive molecular probes on the thermal phase transition in dimyristoylphosphatidylcholine preparations. *Biochim. Biophys. Acta.* 944:164–176.
22. Bammel, B. P., D. D. Hamilton, R. P. Haugland, H. P. Hopkins, J. Schuette, W. Szalecki, and J. C. Smith. 1990. NMR, calorimetric, spin-label, and optical studies on a trifluoromethyl-substituted styryl molecular probe in dimyristoylphosphatidylcholine vesicles and multilamellar suspensions: a model for location of optical probes. *Biochim. Biophys. Acta.* 1024:61–81.
23. Bae, S. J., S. Kitamura, L. G. Herbette, and J. M. Sturtevant. 1989. The effect of calcium channel blocking drugs on the thermotropic behavior of dimyristoylphosphatidylcholine. *Chem. Phys. Lipids.* 51:1–7.

24. Constantinides, P. P., L. Ghosaini, N. Inouchi, S. Kitamura, R. Seshadri, M. Israel, A. C. Sartorelli, and J. M. Sturtevant. 1989. Interaction of *N*-alkylanthracyclines with lipid bilayers: correlation between partition coefficients, lipid phase distributions and thermotropic behavior. *Chem. Phys. Lipids.* 51:105–118.

25. Sturtevant, J. M. 1984. The effects of water-soluble solutes on the phase transition of phospholipids. *Proc. Natl. Acad. Sci. USA.* 81:1398–1400.

26. Simon, S. A., T. J. McIntosh, and M. L. Hines. 1986. The influence of anesthetics on the structure and thermal properties of saturated lecithins. *In* Molecular and Cellular Mechanism of Anesthetics. S. H. Roth, and K. W. Miller, editors. Plenum Publishing Corp., New York. 297–308.

27. Anderson, O. S., S. Feldberg, H. Nakadomari, S. Levy, and S. McLaughlin. 1978. Electrostatic interactions among hydrophobic ions in lipid bilayer membranes. *Biophys. J.* 21:35–70.

28. Haverstick, D. M., and M. Glaser. 1987. Visualization of Ca^{+2}-induced phospholipid domains. *Proc. Natl. Acad. Sci. USA.* 84:4475–4479.

29. Haverstick, D. M., and M. Glaser. 1989. Influence of proteins on the reorganization of phospholipid bilayers into large domains. *Biophys. J.* 55:677–682.

30. Vaz, W. L. C., E. C. C. Melo, and T. E. Thompson. 1989. Translational diffusion and fluid domain connectivity in a two-component, two-phase phospholipid bilayer. *Biophys. J.* 56:869–876.

31. Vaz, W. L. C., E. C. C. Melo, and T. E. Thompson. 1990. Fluid phase connectivity in an isomorphous two-component, two-phase phosphatidylcholine bilayer. *Biophys. J.* 58:273–275.

32. Melchior, D. L. 1986. Lipid domains in fluid membranes: a quick-freeze differential scanning calorimetry. *Science (Wash. DC).* 234:1577–1580.

33. Luo, C., C. Zhu, L. Ruan et al. 1990. Scanning tunneling microscopy of the phosphatidylcholine bilayer. *J. Vac. Sci. Technol.* A8:684–697.