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Adsorption of ionized and neutral pentachlorophenol to phosphatidylcholine membranes

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We have studied adsorption of pentachlorophenol (PCP) to phosphatidylcholine (PC) membranes by measuring the electrophoretic mobility of multilayered lipid vesicles in PCP solutions. PC vesicles become negatively charged due to the adsorption of ionized PCP, and we have found that their zeta potential depends upon the ionic strength and pH of the aqueous suspension. We have shown that the experimental results can be adequately accounted for in terms of a two-component Langmuir-Stern-Grahame adsorption model assuming that the 'PCP adsorption sites' are occupied either by the neutral (HA) or the ionized (A^-) species. The characteristics of adsorption isotherms of the PCP - PC membrane are as follows: the association constants are $K_A = 55000\ dm^3/mol$, $K_{HA} = 279000\ dm^3/mol$; 4.3 PC molecules make up each PCP adsorption site at saturation; the linear partition coefficients are $\beta_{HA} = (15.5 \pm 0.7) \cdot 10^{-5}$ m and $\beta_A = (3.0 \pm 0.3) \cdot 10^{-5}$ m. The properties of PCP adsorption isotherms for PC membranes predict an increased pK_a value of membrane-bound PCP, which has been observed in related studies.

Introduction

At the center of interest in membrane biophysics and biochemistry is an understanding of proton transport [1–3] and the interactions of weak organic acids with biomembranes, specifically energy transducing biomembranes [4–8]. Weak organic acids are biologically active as uncouplers of oxidative phosphorylation and photophosphorylation because of their ability to induce proton permeability in membranes. The increased membrane electrical conductivity associated with this protonophoretic effect has been used very effectively in studies of the mechanism of proton transport in membranes [9–16].

McLaughlin and Dilger [17] have shown that a good correlation exists between uncoupling activity in mitochondria and an increase in membrane electrical conductivity when the uncoupler concentration is low. At higher uncoupler concentrations other mechanisms alter the biomembrane function. Reyes and Benos [18] have shown that, in the case of carbonylcyanide phenylhydrazone uncouplers, the inhibition of oxygen uptake observed at higher concentrations may be related to a change in membrane interfacial potential difference. From this it can be seen that an understanding of the biological activity of weak organic acids requires knowledge of their adsorption characteristics with biomembranes. In this paper we provide such data for pentachlorophenol.

Pentachlorophenol (PCP) is a weak acid (pK_a

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= 4.75) [19] that is acutely toxic to all forms of life. It has been shown that its biological activity is, at least in a part, associated with uncoupling of phosphorylation [20,21].

In addition to the biophysical and biochemical significance of studies of PCP–membrane interactions, there is a strong ecotoxicological interest in this compound. PCP has been used for decades as a major pesticide, disinfectant and wood preservative due to its potent biocidal activity. It has been estimated that world-wide yearly production is $5 \cdot 10^7$ kg and that it has polluted various ecosystems [22]. Though PCP is degradable, estimates of PCP concentrations in human adipose tissue range from 24 μg of PC/kg in the Federal Republic of Germany to 140 μg/kg in Japan [23].

The structural complexities of biomembranes necessitate studies of uncoupler interactions with lipid bilayer membranes. This is the second paper in a series of detailed studies of pentachlorophenol/membrane interactions. In the earlier work [24] we gave estimates of the dielectric constant of the PCP adsorption/ionization sites in PC and PG membranes and the pK_a of PCP adsorbed onto lipid bilayers. In this paper we report on our studies of the electrophoretic mobility of lipid vesicles as a function of PCP concentration, pH and ionic strength from which we have determined the parameters of Langmuir-Stern-Grahame adsorption isotherms.

Materials and Methods

Potassium chloride (Baker Chemicals, Phillipsburg, NJ), potassium phosphate dibasic trihydrate, potassium citrate monohydrate and boric acid (Mallinckrodt Chemicals, St. Louis, MO) were analytical grade. Water was purified with Milli-Q Water Purification System (Millipore Corp., Bedford, MA). Potassium chloride and buffer solutions were uniformly prepared to minimize systematic errors. Pentachlorophenol (PCP) (Aldrich Chemical Co., Milwaukee, Wis.) was dissolved in potassium hydroxide solution (pH = 12) to make stock solutions in the concentration range 1–4 mM. The concentration was checked spectrophotoscopically using a Beckman DU-7 spectrophotometer. The pentachlorophenol testing solutions were prepared by quantitative dilution of the stock solution with buffer or a salt and buffer combination. Egg phosphatidylcholine (PC) and phosphatidylglycerol (PG) in chloroform solution were obtained from Avanti Polar Lipids, (Birmingham, AL). The phospholipid concentrations in stock solutions were checked periodically because of evaporation of chloroform with use.

Multilamellar vesicles for electrophoretic measurements were prepared by a method similar to that of Bangham et al. [25]. Phospholipids were diluted with chloroform (lipid : chloroform, 1 mg : 200–300 ml) in a round bottom flask using a volume greater than twice that of the final solution (e.g. for 10 ml of vesicle suspension, a 25 ml flask was used). Chloroform was removed by rotary flash evaporation using a Flash-Evaporator (Buchler Instruments, Fort Lee, NJ). The round bottom flask containing the lipid film was then purged with dry nitrogen to ensure complete removal of chloroform. Buffer solution was added and the flask was manually shaken to suspend the lipid. The lipid/chloroform dilution ratio, speed, intensity and time of shaking were factors aiding the formation of uniformly sized vesicles.

Samples were prepared by mixing the aqueous buffer solution (with or without salt) with the pentachlorophenol solution and the vesicle suspension. High pH (> 7) samples were stored under nitrogen.

Electrophoretic mobilities were measured using a Rank Brothers Mark I microelectrophoretic instrument (Bottisham, Cambridge, U.K.). The current was monitored and the electrodes were well plated to ensure that no significant polarization occurred. The cylindrical measuring cell was cleaned with 1 : 1 chloroform/methanol mixture or chromic-sulfuric acid cleaning solution. Using the latter required thorough rinsing to ensure that the cell was free of chromium. The position of the stationary level was checked frequently.

The mobility measurements for pH and ionic strength dependence experiments were made at 20 °C.

Electrophoretic mobility of each sample was obtained as the average of approx. 30 measurements, with alternating polarity, at various depths of focus of the microscope in the vicinity of the stationary layer. The electrophoretic mobility was determined from a least-squares fit of the velocity

of vesicles versus the distance from the stationary layer.

Adsorption isotherms were obtained from the electrophoretic mobility of vesicles charged by the adsorption of pentachlorophenolate ions. The Helmholtz-Smoluchowski equation was used to determine ζ-potentials from the electrophoretic mobility, μ.

$$\mu = \varepsilon\varepsilon_0\zeta/\eta \tag{1}$$

where η is the viscosity, ε is the dielectric constant of the solution, and ε_0 is the permittivity of free space. The density of ionized PCP adsorbed to the membrane was obtained from the charge density using the diffuse double layer model of the membrane/water interface.

Adsorption characteristics defined in the model below were obtained by a nonlinear least-squares fit procedure in which chi-squared was minimized using a gradient expansion algorithm described by Bevington [26]. The uncertainties of the adsorption parameters were obtained as the square root of the diagonal elements of the error matrix.

Prototype adsorption model

The treatment of pentachlorophenol (HA) and pentachlorophenolate (A^-) adsorption is based on the Langmuir-Stern-Grahame model [27]. This model has been found to be applicable to the adsorption of a great variety of ions onto electrically neutral and charged membranes [28–32].

The present prototype model includes these major features: (a) The saturation density of binding sites is assumed to be an adjustable quantity that is less than the density of lipids, and (b) the binding sites are common for both HA and A^- species. The prototype adsorption model can be summarized as follows. The density of adsorption sites, Q, is proportional to the surface density of membrane lipids, (L),

$$Q = \phi(\mathrm{L})/N_A \tag{2}$$

the physical meaning of the quantity ϕ is that its reciprocal value, $1/\phi$ gives the number of lipids per adsorption site, N_A, the number of Avogadro. The value of (L) used corresponds to 0.7 nm^2 per lipid molecule [33]. The coverages of membrane surface, θ, for the ionized and the neutral species are, respectively,

$$\theta_A = (A^-)_m/Q \tag{3}$$

and

$$\theta_{HA} = (HA)_m/Q \tag{4}$$

The condition for competitive adsorption is

$$\theta_A + \theta_{HA} + \theta_{free} = 1 \tag{5}$$

Adsorption isotherms are given by

$$(1 + K_A[A^-]_m)\theta_A + K_A[A^-]_m\theta_{HA} = K_A[A^-]_m \tag{6}$$

and

$$K_{HA}[HA]_m\theta_A + (1 + K_{HA}[HA]_m)\theta_{HA} = K_{HA}[HA]_m \tag{7}$$

where the square brackets indicate aqueous concentrations and the parentheses surface densities ($(HA)_m$, the membrane surface density of neutral PCP). K_A and K_{HA} are the intrinsic association constants characterizing the membrane-PCP system. In view of the small values of ζ-potential for uncharged vesicles in the absence of PCP, the adsorption of salts and buffer components has been omitted.

For charged membranes, due to either the charged lipids or adsorbed A^-, the concentration of A^- at the aqueous side of the membrane/water interface is given by

$$[A^-]_m = [A^-]_0 \exp(qV_m/kT) \tag{8}$$

whereas for the neutral molecules

$$[HA]_m = [HA]_0 \tag{9}$$

The subscript 0 indicates the bulk aqueous concentration, q is the charge of A^- and is equal to $-e$, V_m is the electric potential difference between the aqueous side of the membrane/water interface, and the bulk solution and k is Boltzmann's constant.

For membranes made of uncharged lipids, such as egg phosphatidylcholine (PC), the electroneutrality condition for the membrane/water interface is

$$\sigma_m(\text{adsorbed } A^-) + \sigma_{aq}(\text{diffuse layer}) = 0 \tag{10}$$

indicating that the membrane surface charge is compensated by the space charge of opposite polarity in the aqueous phase. The membrane surface potential, which is equal to V_m, is obtained from Grahame's equation

$$\sigma_m^2 = 2kT\varepsilon\varepsilon_0 \sum_i C_i[\exp(\nu_i q V_m/kT) - 1] \quad (11)$$

where C_i is the volume density of salt and buffer ions of valency ν_i.

The ζ-potential is the electric potential at a distance s from the membrane surface, $V(s)$. It can be related to the membrane surface potential according to

$$\zeta = V(s) \quad (12.1)$$

or approximated by

$$\zeta = V_m \exp(-s/d) \quad (12.2)$$

where d is the Debye length parameter. Numerical integration of the Poisson-Boltzmann equation for the case of a multivalent buffer was done according to a method proposed by Bentz and Nir [34] to obtain s, the position of the shear surface.

Other quantities of interest are the linear partition coefficients, β_A and β_{HA}, relating both the membrane density and the aqueous concentration of the ionized and neutral acid at low surface coverages (Henry's approximation),

$$\beta_A = K_A Q \quad (13)$$

and

$$\beta_{HA} = K_{HA} Q \quad (14)$$

Results and Discussion

Location of the shear surface

By definition, the ζ-potential measures the electric potential difference between the shear envelope of the particle and the bulk aqueous phase. The location of the shear plane with respect to the particle surface is an elusive quantity which is not precisely known. Carrol and Haydon [35] have shown for example, how the position of the shear surface is dependent on the size of polar head groups in surfactants. McLaughlin and co-workers [28,32] have done detailed studies of the electric potential distribution at the membrane/water interface. From a combination of ζ-potential and membrane surface potential measurements using various methods, they inferred that in 0.1 M NaCl solutions the shear surface is 0.2 nm away from the membrane surface.

We have made independent measurements of shear plane location; because of our need to work at various pH values, we have used a more complex buffer containing multivalent ions. The position of the shear surface was obtained by measuring the ζ-potential of vesicles prepared from a mixture of negatively charged PG and neutral PC lipids and then determining the distance at which $(V(x) = \zeta$ by integrating the Poisson-Boltzmann equation. We have used vesicles containing 10 and 30 mol% PG, so that the magnitude of ζ-potentials is comparable to that obtained in the PCP adsorption studies. The position of the shear surface is regarded as an empirical parameter.

Fig. 1 illustrates the dependence of shear surface position as a function of ionic strength. Since potassium is known to adsorb to the negatively

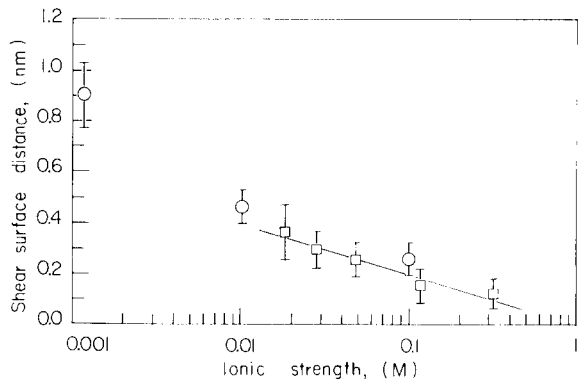

Fig. 1. Calibration of the shear surface position by equating the experimental ζ-potential to that predicted by the diffuse double layer model of the membrane/water interface for negatively charged membranes prepared from a mixture of PC and PG. Squares, present work: The aqueous phase contained phosphate/citrate/borate buffer (0.002/0.002/0.0005 M), pH = 7.5. Ionic strength was controlled by varying the concentration of KCl. The least-squares fit line was used to obtain the shear surface distance as a function of ionic strength in studies of adsorption of ionized pentachlorophenol. Circles, the shear surface distance computed from data given in Ref. 31.

charged PG, its adsorption was taken into account and the association constant was set to 0.15 dm^3/mol according to studies [28]. Our results are denoted by squares; circles indicate the position of the shear surface computed from ζ-potential data for PS/PC membranes in tetramethylammonium chloride solutions given in appendix A of Ref. 32. The results suggest that within the range of moderately strong electrolyte concentration, the shear surface distance logarithmically decreases with the increase of ionic strength.

Binding of pentachlorophenolate to PC membranes

Effect of ionic strength

At pH $\gg$ pK_a(PCP) the concentration of neutral PCP is negligible so that the ζ-potential of vesicles provides information on the membrane/A^- adsorption characteristics only. We have measured the PCP concentration dependence of ζ-potential as a function of KCl concentration at pH = 10. The KCl concentrations were 0, 0.002, 0.004, 0.006, 0.01, 0.016, 0.03, 0.06, 0.1, 0.2 and 0.3 M. The results for three KCl concentrations are shown in Figs. 2a and b. They are: low 0.002 M, intermediate 0.03 M and high 0.3 M. The semilogarithmic plot in Fig. 2a shows the onset of A^- adsorption, which occurs at micromolar PCP concentrations. The linear plot, Fig. 2b, is useful for inspecting the saturation portion of the adsorption isotherm.

The effect of aqueous phase ionic strength is expected to be two-fold: (1) alteration of the energeetics of adsorption, and (2) screening of the charged membrane surface. The model (Eqns. 2–14) makes it possible to discriminate between these two effects.

The adsorption characteristics, i.e., association constant, K_A, and the membrane surface saturation factor, ϕ, were obtained by a two-parameter fit and the linear partition coefficient, β_A, was calculated according to Eqn. 13. The data set included the ζ-potential-PCP aqueous concentration pairs for all KCl concentrations mentioned earlier. The respective values were: $K_A = (0.70 \pm 0.05) \cdot 10^5$ dm^3/mol, $\phi = 0.170 \pm 0.006$ and $\beta_A = (2.8 \pm 0.21) \cdot 10^{-5}$ m.

We have not found any significant effect of ionic strength on the adsorption characteristics,

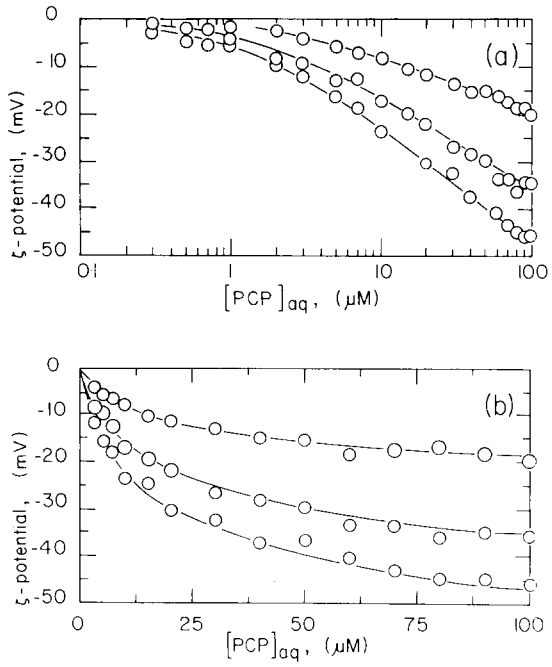

Fig. 2. Dependence of the ζ-potential of egg PC vesicles on PCP aqueous concentration at pH = 10, corresponding to fully ionized PCP. (a) Semilogarithmic plot illustrating the onset of PCP adsorption; (b) linear plot depicting the membrane surface saturation effect. The aqueous phase contained phosphate/citrate/borate buffer (0.002/0.002/0.0005 M) and 0.002 M KCl for the lower data set, 0.03 M for the middle data set, and 0.3 M for the upper data set. The solid curves are predictions of the adsorption model for the association ($K_A = 70000$ dm^3/mol) and surface saturation ($\phi = 0.17$) constants.

suggesting that the screening of the membrane surface by ions has little bearing on the energetics of adsorption. This type of behavior is consistent with adsorbed pentachlorophenolate located at some depth below the membrane surface. In this case, the repulsive $A^- \ldots A^-$ interactions would be mediated primarily by the membrane phase with the ion-screening effects being energetically insignificant.

Effect of pH on the ζ-potential

Figs. 3a and b show the variation of ζ-potential as a function of PCP concentration at different pH values. The main feature in the data is that the magnitude of ζ-potential decreases as the pH is lowered and that the saturation level decreases as well. The latter feature indicates that membrane coverage by the neutral form inhibits binding of the ionized species.

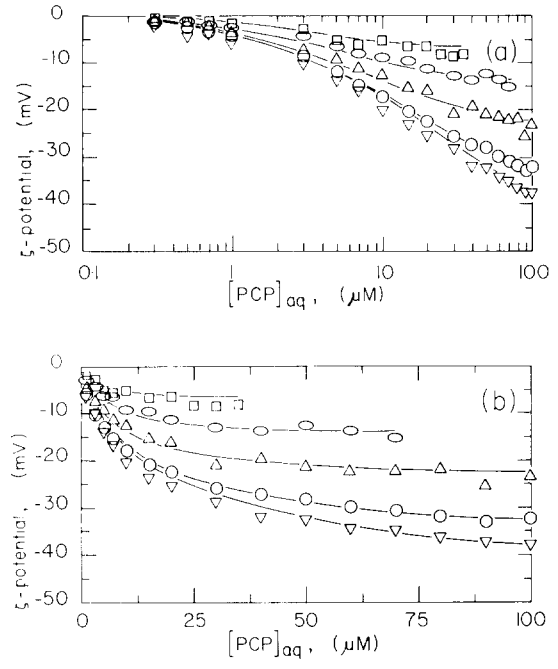

Fig. 3. Effect of pH on the ζ-potential of egg PC vesicles in aqueous PCP solution. (a) Semilogarithmic plot depicting the onset of PCP adsorption; (b) linear plot depicting the membrane surface saturation of PCP. The aqueous phase contained phosphate/citrate/borate buffer (0.002/0.002/0.0005 M) and 0.03 M KCl, pH values, from the top down, are: 4.5, 5.0, 5.5, 6.2 and 7.3. The solid curves illustrate the adsorption model using the characteristics given in Table I for the shear surface distance computed from calibration data given in Fig. 1.

The experimental results were analyzed in terms of the 2-component adsorption model described earlier. Three membrane parameters (K_A, K_{HA} and ϕ), obtained by minimizing chi-squared, are given in Table I. In this analysis, we have included ζ-potential-PCP aqueous concentration pairs for pH values of 4.5, 5.0, 5.5, 6.2 and 7.3 at a KCl concentration of 0.03 M. In row (a) are the results obtained under the assumption that the shear surface is located at a distance of 0.255 nm from the membrane surface. Row (b) lists the results using a shear surface distance equal to zero, which is equivalent to setting the membrane surface potential equal to the ζ-potential. The major effect of the shear surface location is on K_A, which is greater using the latter assumption, since the ζ-potential underestimates the value of membrane surface potential. K_{HA} was unaffected by the selection of shear surface distance.

TABLE I

ADSORPTION CHARACTERISTICS OF EGG PHOSPHATIDYLCHOLINE MEMBRANES AND OF NEUTRAL (HA) AND IONIZED (A^-) PENTACHLOROPHENOL

Phosphate/citrate/borate buffer (0.002/0.002/0.0005 M) [KCl] = 0.03 M.

Assumed position of shear surface s (nm)	Associated constant (10^5 dm^3/mol)		Membrane surface saturation
	K_{HA}	K_A	
0.255	2.8±0.1	0.55±0.05	0.23±0.02
0.0	2.8±0.1	0.77±0.05	0.14±0.01

It is noteworthy that the value of the membrane saturation parameter, ϕ, which is related to the density of PCP adsorption sites (Eqn. 2), when derived from the pH effect studies was found to be greater than that obtained from the ionic strength studies *. This result suggests that the saturation density of adsorption sites for the HA form is greater than that for A^-. The difference in the magnitude of the saturation parameter in the above two cases can be regarded as a manifestation of the long range repulsive $A^- \ldots A^-$ coulombic force between membrane bound A^- molecules that determines, among other factors, the saturation density of A^-. The greater value of ϕ obtained at low pH may be assigned to the absence of coulombic repulsion between the membrane bound HA molecules.

The energetics of PCP adsorption to PC membranes is schematically depicted in Fig. 4. The change in free energy on transfer of PCP from water, assuming a 0.4 nm adsorption layer [36], is −0.29 eV for A^- and −0.33 eV for HA. The neutral PCP molecules are stabilized in the membrane to a greater degree than their anions. This is due in part to the low dielectric constant for the PCP adsorption/ionization site reported in [24].

* Using Student's T-test, the ϕ value calculated when ionized PCP was present alone was different from that obtained when ionized and unionized PCP both were present at a confidence level greater than 99%.

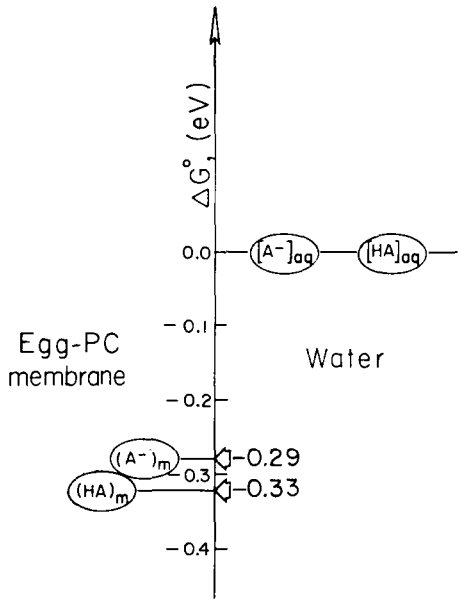

Fig. 4. Schematic diagram depicting the energetics of pentachlorophenol adsorption to egg PC membranes as derived from association constants K_{HA} and K_A. Typically, for pentachlorophenol, the neutral species is stabilized on adsorption to egg PC membranes. The thermal energy involved, kT, is 0.025 eV.

Conclusions

Within the framework of our adsorption model, the density of ionized and neutral PCP at low membrane surface coverage is approximately given by

$$(A^-)_m = \beta_A [A^-]_0 \quad (15)$$

and

$$(HA)_m = \beta_{HA} [HA]_0 \quad (16)$$

The apparent dissociation constant of membrane bound PCP can be related to its standard aqueous value and the adsorption characteristics by

$$K_a(m) = [H^+](A^-)_m/(HA)_m \quad (17)$$

Due to a greater value of the association constant of the neutral species (see Table I) the surface pK_a of PCP is shifted from its standard aqueous value of 4.75 [19]. The pK_a shift is

$$\Delta pK_a = pK_a(m) - pK_a(aq) = \log_{10}(K_{HA}/K_A) \simeq 0.7 \quad (18)$$

This value is in a fairly good agreement with the pH shifts of membrane conductivity maxima from the pK_a of PCP, $\Delta pK_a(cond) = 0.5$ and pK_a changes of membrane-bound PCP obtained by ultraviolet spectrophotometry, $\Delta pK_a = 1$, reported recently in [24].

In general, taking into account the adsorption isotherms for HA and A^- the two-component adsorption model predicts that the apparent membrane pK_a of adsorbed PCP depends on the PCP aqueous concentration and the ionic strength. The model predictions based on values of adsorption parameters obtained in this study are shown in Fig. 5. The lower curve gives the pK_a dependence of membrane bound PCP predicted for the experimental conditions of high buffer concentration used in the studies of pH dependence of PCP-induced membrane conductivity [24]. The triangle indicates the pK_a value of membrane bound PCP corresponding to the maximum of membrane conductivity in that study. The upper curve illustrates the increase in the pK_a value of membrane-bound

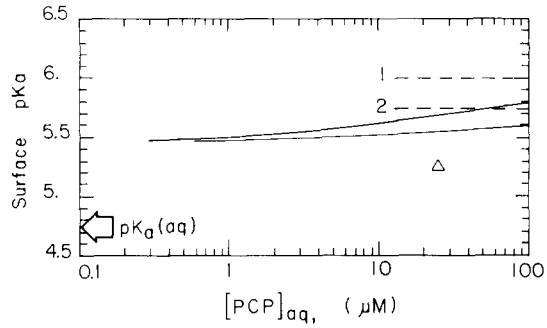

Fig. 5. The pK_a dependence of pentachlorophenol, adsorbed to egg PC membranes, on PCP aqueous concentration and the solution ionic strength as predicted by the adsorption model using the association constants given in Table I. The lower curve corresponds to high ionic strength conditions used in membrane conductivity studies [24] (0.1 M KCl and phosphate/citrate/borate buffer 0.2/0.2/0.05 M), and the triangle symbol gives the membrane surface pK_a estimated from those studies. The upper curve corresponds to conditions used in the spectrophotometric determination of the pK_a [24] (0.1 M KCl and phosphate/citrate/borate buffer 0.02/0.02/0.005 M), and the broken lines indicate the apparent pK_a determined spectrophotometrically for PC (1) and PC + cholesterol vesicles (2) (data from Ref. 24). The pK_a of adsorbed PCP predicted from the electrophoretic data and the estimates of surface pK_a from unrelated experiments are all above the standard aqueous value of PCP [19].

TABLE II

PARTITION COEFFICIENTS OF SEVERAL WELL-KNOWN UNCOUPLERS

CCCP, carbonylcyanide *m*-chlorophenylhydrazone
FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone
DTFB, 5,6-dichloro-2-trifluoromethylbenzimidazole
TTFB, 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole
S-13, 5-chloro-3-*tert*-butyl-2′-chloro-4′-nitrosalicylanilide

Uncoupler	Ref.	β_A (10^{-5} m)	β_{HA} (10^{-5} m)	$(pK_a)_m - (pK_a)_{aq}$
CCCP	10	2	2	0
CCCP	11	2.6	–	–
FCCP	12	3	3	0
DTFB, TTFB	13	4	4	0
PCP	this work	3	15	0.7
S-13	9	20	500	1.4

PCP under the conditions used in spectrophotometric pK_a determinations [24]. The horizontal lines indicate the experimental pK_a values obtained for pure PC and cholesterol (30 mol%) containing membranes *. The pK_a values from membrane dissociation and membrane conductivity studies are in qualitatively agreement with the surface pK_a.

The significance of the results reported here is that the two-component Langmuir-Stern-Grahame model, based on the diffuse double layer theory of the aqueous portion of the membrane/water interface, adequately describes adsorption of pentachlorophenol to neutral PC membranes. The quantitative data on the distribution of PCP between water and membranes are useful for the development of an understanding of the intricate relationship between membrane activity and molecular structure of uncouplers of oxidative phosphorylation.

In Table II we compare results of recent studies of uncoupler adsorption to PC membranes. The data suggest that with regard to adsorption to membranes, PCP is an intermediate compound with characteristics in between the well known uncouplers such as CCCP, FCCP and S-13. The partition coefficient of ionized PCP is comparable to those determined for CCCP [10] and FCCP [12]. In contrast to CCCP, FCCP, DTFB and TTFB [13], the neutral form of PCP exhibits a greater affinity to membranes, and the membrane surface pK_a is shifted from the aqueous value. S-13 exhibits the largest values of partition coefficients and the largest pK_a shift [9]. It is interesting that CCCP, FCCP and S-13 are class 1 uncouplers whereas DTFB, TTFB and PCP are in class 2.

It is hoped that the availability of adsorption isotherms for PCP with PC membranes will be useful in unraveling the multicomponent binding observed for phenolic herbicides and biomembranes [37,38], the distribution of PCP between organs in men [23] and the mechanism of action of pesticides at the membrane level [39–41].

* The experimental pK_a values are given by the horizontal lines because adsorption of PCP to vesicles leaves the aqueous concentration in doubt.

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References

1 Ferguson, S.J. (1985) Biochim. Biophys. Acta 811, 47–95
2 Westerhoff, H.V., Melandri, B.A., Venturoli, G., Azzone, G.F. and Kell, D.B. (1984) Biochim. Biophys. Acta 768, 257–292
3 Nagle, J.F. and Dilley, R.A. (1986) J. Bioenerg. Biomembranes 18, 55–64
4 Terada, H. (1981) Biochim. Biophys. Acta 639, 225–242
5 Katre, N.V. and Wilson, D.F. (1977) Arch. Biochem. Biophys. 184, 578–585
6 Terada, H. (1975) Biochim. Biophys. Acta 387, 519–532
7 Wilson, D.F., Ting, H.P. and Koppelman, M.S. (1971) Biochemistry 10, 2897–2902
8 Bakker, E.P., Arents, J.P., Hoebe, J.P.M. and Terada, H. (1975) Biochim. Biophys. Acta 387, 491–506
9 Kasianowicz, J., Benz, R. and McLaughlin, S. (1987) J. Membrane Biol. 95, 73–89
10 Kasianowicz, J., Benz, R. and McLaughlin, S. (1984) J. Membrane Biol. 82, 179–190
11 O'Shaughnessy, K. and Hladky, S.B. (1983) Biochim. Biophys. Acta 724, 381–387
12 Benz, R. and McLaughlin, S. (1983) Biophys. J. 41, 381–398
13 Dilger, J.P. and McLaughlin, S. (1979) J. Membrane Biol. 46, 359–384
14 Smejtek, P., Jayaweera, R. and Hsu, K. (1983) J. Membrane Biol. 76, 227–234
15 Smejtek, P., Hsu, K. and Perman, W.H. (1976) Biophys. J. 16, 319–336

16 Pickar, A.D. and Hobbs, J. (1982) Biochim. Biophys. Acta 93, 221–236
17 McLaughlin, S.A. and Dilger, J.P. (1980) Physiol. Rev. 60, 825–863
18 Reyes, J. and Benos, D.J. (1984) Membrane Biochem. 5, 243–268
19 Drahonovsky, J. and Vacek, z. (1971) Coll. Czech. Chem. Commun. 36, 3431–3440
20 Weinbach, E.C. and Garbus, J. (1965) Biol. Chem. 240, 1811–1819
21 Senger, H. and Raul, D. (1980) Int. J. Biochem. 12, 1045–1048
22 Crosby, D.G. (1981) Pure Appl. Chem. 53, 1051–1080
23 Geyer, H.J., Scheunert, I. and Korte, F. (1987) Chemosphere 16, 887–899
24 Smejtek, P., Barstad, A.W. and Hsu, K. (1987) Biochim. Biophys. Acta 902, 109–127
25 Bangham, A.D., Hill, M.W. and Miller, N.G.A. (1974) Methods Membrane Biol. 1, 1–68
26 Bevington, P.R. (1969) Data Reduction and Error Analysis for the Physical Sciences, pp. 235–245, McGraw-Hill, New York
27 McLaughlin, S. and Harary, H. (1976) Biochemistry 15, 1941–1948
28 Eisenberg, M., Gresalfi, T., Riccio, T. and McLaughlin, S. (1979) Biochemistry 18, 5213–5223
29 McLaughlin, S., Murine, N., Gresalfe, T., Vaio, G. and McLaughlin, A. (1981) J. Gen. Physiol. 77, 445–473
30 Alvarez, O., Brodwick, M., Latorre, R., McLaughlin, A., McLaughlin, S. and Szabo, G. (1983) Biophys. J. 44, 333–342
31 Cohen, J.A. and Cohen, M. (1981) Biophys. J. 36, 623–651
32 McLaughlin, A., Eng, W.K., Vaio, G., Wilson, T. and McLaughlin, S. (1983) J. Membrane Biol. 76, 183–193
33 Small, D. (1986) Handbook of Lipid Research, Vol. 4, The Physical Chemistry of Lipids, p. 512, Plenum Press, New York
34 Bentz, J. and Nir, S. (1980) Bull. Mathematical Biol. 42, 191–220
35 Carrol, B.J. and Haydon, D.A. (1975) J. Chem. Soc., Faraday Trans. 1 2, 361–377
36 Flewelling, R.F. and Hubbell, W.L. (1986) Biophys. J. 49, 531–540
37 Neumann, W., Lassch, H. and Urbach, W. (1987) Pest. Biochem. Physiol. 27, 189–200
38 Oettmeier, W., Kude, C. and Soll, H. (1987) Pest. Biochem. Physiol. 27, 50–60
39 Moreland, D.E. (1980) Annu. Rev. Plant Physiol. 31, 597–638
40 Moreland, D.E. (1981) Pest. Biochem. Physiol. 15, 21–31
41 Stidham, M.A., Siedow, J.N., McIntosh, T.J., Porter, N.A. and Moreland, D.E. (1985) Biochim. Biophys. Acta 812, 721–730