THE MEMBRANE DIPOLE POTENTIAL IN A TOTAL MEMBRANE POTENTIAL MODEL

Applications to Hydrophobic Ion Interactions with Membranes

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ABSTRACT The total potential energy profile for hydrophobic ion interactions with lipid bilayers can be written as the sum of four terms: the electrical Born, image and dipole contributions, and a neutral energy term. We introduce a specific model for the membrane dipole potential, treating it as a two-dimensional array of point dipoles located near each membrane-water interface. Together with specific theoretical models for the other energy terms, a total potential profile is developed that successfully describes the complete set of thermodynamic parameters for binding and translocation for the two hydrophobic ion structural analogues, tetraphenylphosphonium (TPP^+) and tetraphenyboron (TPB^-). A reasonable fit to the data is possible if the dipole potential energy has a magnitude of 5.5 ± 0.5 kcal/mol (240 ± 20 mV), positive inside, and if the neutral energy contribution for TPP^+ and TPB^- is -7.0 ± 1.0 kcal/mol. These results may also have important implications for small ion interactions with membranes and the energetics of charged groups in membrane proteins.

INTRODUCTION

Hydrophobic ions are a fairly unique class of charged molecules that both bind to and readily translocate across pure lipid bilayers. A dramatic feature of these molecules is that anions bind several orders of magnitude more strongly to and translocate several orders of magnitude more rapidly across lipid bilayers than structurally similar cations. Despite this crucial observation apparent in the earliest hydrophobic ion studies (e.g., 50, 54, 55), the theoretical model of Ketterer, Neumcke, and Läuger (46), which describes how such ions interact with membranes, makes no distinction on the basis of charge. It has been proposed that lipid bilayers possess a substantial membrane dipole potential that is responsible for these differences (e.g., 41, 77, 78; for further historical references see reference 27), but no self-consistent model has yet been developed. The absence of any detailed data on hydrophobic cations is undoubtedly responsible for this state of affairs. To fill this need and thereby permit a detailed comparison of the two ideal structural analogues tetraphenylphosphonium (TPP^+)[1] and tetraphenylboron (TPB^-), the thermodynamic properties of TPP^+ interactions with neutral membranes were first thoroughly studied, as reported in the accompanying article (30).

We are now able to develop here a complete potential profile for hydrophobic ion interactions with lipid bilayers. Following the original theory of Ketterer, Neumcke, and Läuger (46), but now incorporating an explicit term for the membrane dipole potential, the free energy as a function of position z for a hydrophobic ion in an aqueous-lipid bilayer system can be written as the sum of electrical and nonelectrical terms:

$$W_{TOT}(z) = W_B(z) + W_I(z) + W_D(z) + W_N(z). \qquad (1)$$

The Born (W_B), image (W_I), and dipole (W_D) energy contributions contain all of the dominant electrical interactions of a hydrophobic ion with the membrane. They represent the ways in which a charged molecule interacts, respectively, with bulk dielectrics, dielectric interfaces, and any intrinsic dipole potential. The neutral energy term (W_N) includes all of the other contributions to the free energy, such as hydrophobic, van der Waals, and steric factors. Specific chemical interactions are also possible, but since a broad class of hydrophobic ions are observed to have remarkably similar binding and translocation proper-

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[1] *Abbreviations used in this paper*: ANS^-, 1-anilino-8-napthalenesulfonate; $CCCP^-$, carbonylcyanide *m*-chlorophenylhydrazone; DLPE, 2,3-dilauroyl-DL-glycero-1-phosphorylethanolamine; DMPC, 2,3-dimyristoyl-D-glycero-1-phosphorylcholine; DNP^-, 2,4-dinitrophenol; DPA^-, dipicrylamine; EPR, electron paramagnetic resonance; $FCCP^-$, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; LPPC, 3-lauroylpropanediol-1-phosphorylcholine; MOPS, 3-(*N*-morpholino)-propane-sulfonic acid; NMR, nuclear magnetic resonance; PCB^-, phenyldicarbaundecaborane; TNP^-, 2,4,6-trinitrophenol; TPA^+, tetraphenylarsonium; TPB^-, tetraphenylboron; TPC°, tetraphenylmethane; TPGe°, tetraphenylgermanium; TPP^+, tetraphenylphosphonium.

BIOPHYS. J. © Biophysical Society · 0006-3495/86/02/541/12 $1.00
Volume 49 February 1986 541-552

ties, we assume this contribution to be of less importance, an assumption considered further in the Discussion. As the very name "hydrophobic ions" suggests, the dominant neutral energy contribution is the hydrophobic energy, and this energy term is therefore often given this name. To alleviate the difficulties in accounting for such energy, we choose to focus attention on the two nearly identical structural analogues TPP^+ and TPB^-, whose effective radii and neutral energy contributions are expected to be nearly identical, a point considered further in the Discussion.

Our general conclusion is that the more complete potential profile of Eq. 1 that explicitly includes the membrane dipole potential can yield a successful self-consistent account not only for the binding and translocation properties of both anions and cations, but also for individual enthalpic and entropic contributions. The model thus provides a general energetic treatment for hydrophobic ion interactions with membranes. In particular, it is found that a membrane dipole potential of ~ 240 mV, inside positive, can account for the differences in TPP^+ and TPB^- interactions with phosphatidylcholine bilayers. Furthermore, the more realistic treatment of the membrane dipole potential presented here permits a quantitative consideration of this component in a wide range of other membrane electrical phenomena, including small ion binding and translocation, and the energetics of protein charged groups in membranes. Some of the material presented here has already appeared in a preliminary form (25, 26).

THEORY

The various energy terms in Eq. 1, apart from the dipole potential, have appeared previously in the literature (3, 42), but are briefly reviewed here to set up a consistent notation and to point out several important features often overlooked. These terms will then be combined below into a total self-consistent model that explicitly includes the membrane dipole potential.

Born Energy

The free energy of transfer in moving an ion of charge q and radius r from a region of dielectric strength ϵ_2 to a region of dielectric strength ϵ_1, is given by the Born (10) expression (here, and in subsequent equations, the leading quantity in brackets gives the conversion from cgs to SI units):

$$W_B = \left[\frac{1}{4\pi\epsilon_0}\right]\frac{q^2}{2r}\left(\frac{1}{\epsilon_1} - \frac{1}{\epsilon_2}\right). \tag{2}$$

The Born model is relatively successful in accounting for hydration energies if appropriate radii are used and adjustments in the local dielectric constant or specific molecular interactions are considered (for reviews see references 9 and 65). For large ions, such as the hydrophobic ions of interest here, it proves to be sufficient to use the simple Born expression (Eq. 1) and treat the ion radius as a parameter to vary within a narrow range of values, or to be fit by experiment (see especially reference 42). Further justification for such a treatment is provided below.

Image Energy

A lipid bilayer is to a first approximation a thin dielectric slab ($\simeq$ 40 Å) sandwiched between two highly conductive (aqueous) volumes. A charged body that has penetrated into the bilayer a distance greater than its ionic size already has acquired the bulk of its Born energy, and this constitutes the major electrostatic interaction of the ion with the membrane. Because of the relative thinness of the membrane, however, the interactions of the charged body with the interfaces are also important, and this contribution is called the "image energy". Image energy calculations applicable to lipid bilayers have been developed in the literature on several occasions (37, 55, 59, 60); yet it has apparently escaped previous attention that none of these are in agreement (for a detailed comparison see reference 24).

The notation used here is as shown in Fig. 1, where the charge q is in a dielectric slab of thickness d and uniform dielectric constant ϵ_1, at distance x from one interface. The slab is sandwiched on both sides by semi-infinite dielectrics of uniform dielectric constant ϵ_2. The combined Born and image energy solution of Neumcke and Läuger (55), with the definition $\alpha = (\epsilon_2 - \epsilon_1)/(\epsilon_2 + \epsilon_1)$, is then given by:

$$W_{B-I}(x) = W_B - \left[\frac{1}{4\pi\epsilon_0}\right]\frac{q^2}{4\epsilon_1}\left\{\frac{1}{x} + \frac{1}{d}\sum_{n=1}^{\infty}\cdot\left[\frac{\alpha^{2n}}{n + x/d} + \frac{\alpha^{2n-2}}{n - x/d} - \frac{\alpha^{2n}}{n + r/d} - \frac{\alpha^{2n-2}}{n - r/d}\right]\right\}. \tag{3}$$

Different limiting conditions of Eq. 3 are considered in Appendix A, where it is shown that under normal conditions of interest ($r \lesssim 4$ Å, $d \simeq 40$ Å, $r \lesssim x \lesssim d/2$), a convenient Born-image energy expression is simply,

$$W_{B-I}(x) = \left[\frac{1}{4\pi\epsilon_0}\right]\frac{q^2}{2\epsilon_1 r}\left[1 - \frac{r}{2x} - 1.2\left(\frac{r}{d}\right)\left(\frac{x}{d}\right)^2\right]. \tag{4}$$

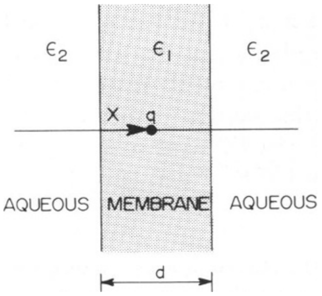

FIGURE 1 Simple lipid bilayer model. A thin dielectric slabof bulk dielectric constant ϵ_1 and thickness d is sandwiched between two bulk (aqueous) dielectric volumes of dielectric constant ϵ_2. A charge q is located a distance x from one interface.

In the conductor limit ($\alpha = 1$) Eq. 4 is good to better than 1%; and for lipid bilayers, where $\epsilon_1 = 2$ and $\epsilon_2 = 78$ ($\alpha = 0.95$), the difference between Eq. 4 and the full theoretical expression, Eq. 3, is rarely more than 4%.

Born-Image Energy Model

In order to incorporate Eq. 4 into the total potential model, we want to write it with respect to the center of the bilayer, z, rather than with respect to the dielectric interface, x. Thus we temporarily introduce t_e as the effective (electrical) bilayer half-thickness; thus $x = t_e - z$ and $d = 2t_e$. Below we show that $t_e = t + h/4$ is the best definition for the effective bilayer half-thickness, where t is approximately the lipid tail length and h is approximately the lipid headgroup thickness (see the dielectric function discussion and Table I). It should also be carefully noted that the Born-image energy formulations become meaningless for distances much less than the ion size. As best seen by Eq. 4, as x takes on values somewhat less than $r/2$, W_{B-I} becomes negative. But this is at spatial scales on the order of angstroms, and on this scale the concept of the dielectric interface loses meaning. Following others who have treated this problem (e.g., 42, 55) it is therefore customary to cutoff the Born-image energy when it becomes zero, and to set it equal to zero everywhere beyond that point. It is also reasonable to create a function that will give the profile a short but smooth transition to zero in this region and thereby eliminate the discontinuity artifact, a procedure that will be followed here (see Appendix B for the "tail" function used).

TABLE I
SUMMARY OF PARAMETERS FOR THE TOTAL POTENTIAL MODEL

Parameter	Expected values	Significance
t	10 – 20 Å	Hydrocarbon (tail) region thickness where $E(z) = \epsilon_1$
h	4 – 10 Å	Dielectric transition (headgroup) region thickness
p	$t - t + h$ Å	Dipole layer location
r	1 – 5 Å	Effective ion radius
a	4 – 9 Å	Effective point dipole separation distance
u	0 – 2 D	Effective point dipole strength
q	e	Ion charge
ϵ_1	2	Membrane dielectric constant
ϵ_2	78	Water dielectric constant
W_N^0	−4 – −8 kcal/mol	Free energy of transfer for neutral molecule

Dipole Energy

When two mobile phases are in contact, it is a general phenomena that a difference of electrical potential is established across the interface. This is a consequence of the intermolecular forces that often act near interfaces to produce selective association of charged molecules, or selective orientation of dipolar molecules. In the absence of strongly adsorbing ions, molecular dipoles are the primary determinants of the surface potential, and a dipole potential at the interface will exist; the only question is its magnitude.

Dipole Potential Concept

Surface chemists and electrochemists in the early part of this century carefully studied the properties of surface potentials and developed an interpretation in terms of molecular dipole layers (see references 2, 19, and 34 for reviews). It was well known, for example, that fatty acid monolayers at air-water interfaces normally have surface potentials of several hundred millivolts, positive on the air side relative to the water. The potential difference across an interface due to a uniformly distributed dipole layer can be approximated by a simple capacitor model. For an effective surface density of molecular dipoles, n (in $Å^{-2}$), each with an effective dipole moment normal to the interface, u (in Debye: 1 Debye = 1 D = 3.336×10^{-30}C − m), and ϵ an effective dielectric constant, the dipole potential can be written:

$$\Delta V_D \simeq 38 \frac{nu}{\epsilon} \text{ V}. \qquad (5)$$

The model is only approximate since the actual interface is a complicated interaction of the various polar groups located there, with the dipole sources being discrete, and the dielectric constant a complicated function on the molecular scale of the interface. Nevertheless, Eq. 5 gives a first approximation to the dipole contribution of the surface potential. Consider a monolayer of fatty acids at an air-water interface with the following parameter values: a surface area per molecule in the tens of $Å^2$ (e.g., 19, 34), a carbonyl group contribution of ~ 2 D (68), a local dielectric constant of ~10, and, say, the carbonyls oriented at 45° on the average. Eq. 5 then gives a dipole potential of ~100–200 mV. It is clear that this simple treatment can account for the fairly large potentials observed in monolayers.

In some of the earliest hydrophobic ion studies, Liberman and Topaly (51) and LeBlanc (45) observed that hydrophobic anions permeate membranes much more readily than structurally similar cations. They reasoned that lipid bilayers must intrinsically be several hundred millivolts positive inside, although they did not propose any source for this potential. The relevance of the dipole potential to these observations was eventually brought to light by Haydon and Hladky (37) and Szabo et al. (71), and tested by the effects of cholesterol (70, 71) and adsorbed dipoles (38).

Source of the Dipole Layer

There are three possible sources for the membrane dipole potential: surface water molecules, lipid headgroups, and lipid carbonyls (e.g., 52, 57). Water molecules have quite a substantial dipole moment, ~1.83 D in the gas phase and probably larger in the liquid (17), which undoubtedly contributes to the interfacial dipole potential. Its contribution is, however, fairly complicated, as indicated by the great disparity in reported values for air-water interface surface potentials, ranging from -500 to $+400$ mV (19). While it could be substantial, its total contribution depends significantly on the perturbing effects of other surface molecules; futhermore, its effects are already included in the water dielectric constant to some extent. Phospholipid headgroups typically exist in charged or zwitterionic forms, and so may contribute to the membrane dipole potential. However, theoretical (24, 32, 62) and physical evidence (13, 36, 67, 69, 73) argue for a headgroup lying flat on the membrane surface. And given that the phosphate groups would most likely be closer to the membrane than the rest of the headgroup, their contribution would more likely make the dipole potential more negative inside rather than positive. The third possible contribution to the membrane dipole potential is from the ester groups that link the two fatty acid chains to the glycerol backbone. Ester groups have dipole moments of ~1.8 D (68), and with two per lipid being located in regions of low dielectric, quite substantial dipole potentials could be produced. Structural evidence argues for ester oxygens pointing toward the aqueous phase (14, 28, 29, 35, 39, 61, 72, 74, 75), making the resulting dipole potential positive inside as observed.

Dipole Model

The evidence presented above strongly supports the existence of a substantial membrane dipole potential. Its source is surely the dipolar organization of molecules at the lipid-water interface, with the most significant contribution probably from the ester linkages between the fatty acid chains and the glycerol backbone, although water organization may also be important. We thus model the dipole potential as arising from a two-dimensional, surface array of point dipole sources located at each membrane surface (Fig. 2). It is convenient to think of these point dipoles as the ester linkages for each fatty acid chain, or simply as an effective point dipole per lipid molecule. There is nothing in the formalism that restricts it so, however, and the model allows for the dipole layer to be located anywhere and have any surface density. Also, while discrete dipoles are assumed, the important quantity is the average dipole moment per unit area, with the discrete nature of the sources providing mainly a bit of realistic structure near the interface.

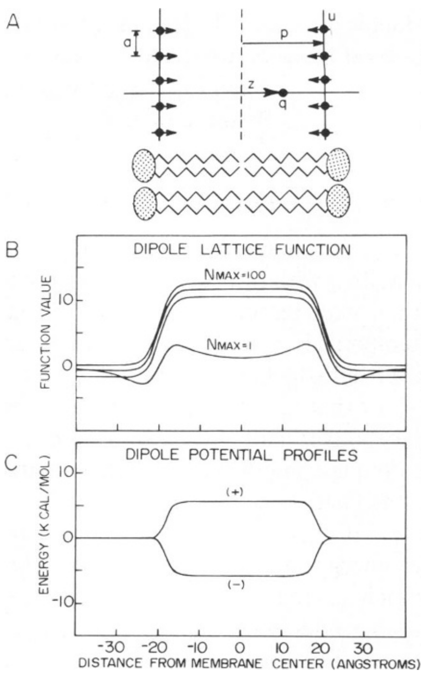

FIGURE 2 Dipole layer model and resulting potential profiles. (*A*) Point dipoles of strength u, directed inward, are separted by a distance a in a two-dimensional square lattice array. Each layer is a distance p, and a charge q is located a distance z, from the bilayer center (q is a distance x from one of the layers). (*B*) Lattice function profiles based on Eq. 8 of the text with the sum taken from one to N_{max}, for $N_{max} = 1$, 10, 50, and 100. (*C*) Resulting dipole potential energy profiles based on Eqs. 6–8 of the text for $q = \pm e$.

In particular, consider a two-dimensional square dipole lattice composed of point dipoles of strength u and separated from one another by a distance a, located near each interface. The two layers are a distance p from the center of the bilayer, with the dipoles (positive ends) pointing inward (Fig. 2). Now consider a charge q a distance z from the center of the bilayer. The goal is to express the potential energy for the charge q as a function of z due to the dipole sheets. It is sufficient to determine the interaction energy of this charge with just one dipole layer, $W_D^1(y)$, where y is the distance from the dipole layer. Since one dipole layer is at $y = p - z$ and the other is at $y = p + z$, the total potential is given by,

$$W_D(z) = W_D^1(p - z) + W_D^1(p + z). \qquad (6)$$

Clearly it is sufficient to consider only positive z since the dipole potential is symmetric about the center of the bilayer for the cases to be considered here (extension of the model to treat asymmetric dipole layers is straightforward and would also be of interest).

To obtain an expression for the energy of the charge at any point, a relatively simple and reasonable case is considered, where the charge is located along a perpendicular coordinate equidistant from the four nearest neighbor point dipoles when it crosses the dipole layer, as indicated in Fig. 2. This choice is supported by the fact that the dipoles are actually physical molecules, such as the lipids, and that due to steric factors, the pathway must be between the molecules. Other calculations are possible but not of substantive value. One could, for example, use some other lattice configuration, such as hexagonal, and also consider charges that do not pass equidistant between individual dipoles. However, preliminary studies in this direction indicate that the loss in simplicity and clarity is not retrieved in predictive ability, given the various other approximations inherent in the model (24).

A dielectric constant as a function of distance from the membrane center, $\epsilon(z)$ (discussed in the following section), is also included. This is clearly only an approximation, but the full description of a dipole layer in a region of variable dielectric is quite complicated, and the introduction of an effective dielectric constant at each point is a reasonable approach. The energy expression for a single dipole layer then follows from the geometry of the problem,

$$W_{\mathrm{D}}^{1}(y) = \left[\frac{1}{4\pi\epsilon_0}\right]\frac{qu}{a^{2\epsilon(z)}}\,L_3(v), \tag{7}$$

where $L_3(v)$ is a three-dimensional lattice function summed-up over the two-dimensional dipole layer with respect to any position y perpendicular to the layer:

$$L_3(v) = 32v\sum_{n=1}^{\infty}\sum_{m=1}^{n} \cdot\left[(2v)^2 + (2m-1)^2 + (2n-2m+1)^2\right]^{-3/2}. \tag{8}$$

Note that the charge-dipole interaction energy varies as $1/r^3$, whereas the number of dipoles in the sheet is proportional to r^2. Each dipole layer out from the origin thus contributes an energy proportional to $1/r$, so the sum over the infinite sheet actually blows up: $\Sigma_{n=1}^{\infty}(1/n) \longrightarrow \infty$. To prevent this, the sum is only carried out to a fixed number of dipole layers, $N_{\max}$, which is justified on both practical and theoretical grounds. Practically, the dipole layer is not flat and infinite, but is limited by the curvature or "coherence length" of the vesicle or membrane sheet. And theoretically, the sum $\Sigma_{n=1}^{\infty}(1/n)$ is very slowly diverging. The nth dipole layer is approximately a distance na away, and for $a \simeq 8$ Å, values of $N_{\max}$ between 10 and 100 correspond to distances of ~80–800 Å. A "coherence" distance within this range is quite reasonable, and it is found numerically that maximum values of the sum (Eq. 8) differ by <10% for $N_{\max}$ changing from 10 to 100, and differ by <4% for $N_{\max}$ changing from 50 to 100 (see Fig. 2).

Dielectric Constant

Most oils and aliphatic hydrocarbons have dielectric constants in the range 2–5, with a value of ~2 generally accepted for the hydrocarbon portion of lipid bilayers, as indicated by impedance measurements of lipid mixtures (23, 33), optical measurements of planar bilayers (56), and a critical comparison of the two methods (20). Low-frequency impedances measurements are consistent with hydrocarbon dielectric values of ~2–3, and they further suggest that the headgroup and ester-group regions have effective dielectric values in 20–50 range, with a value of ~30 being a reasonable choice (5, 6). Effective headgroup dielectric constants have also been extracted from spectrometric properties of amphiphiles, with similar results. The dye ANS$^-$ has been localized by x-ray diffraction (47, 48) and NMR (21, 63) to be in the lipid headgroup region, and its spectroscopic properties are consistent with an effective dielectric environment of ~30 (66). Similar studies with merocyanine 540 indicated a dielectric value of ~8 in the glycerol backbone region (46), and electrostatic surface potential effects have been interpreted in terms of a surface dielectric of ~30 (15, 22). These experimental, and other theoretical (24), considerations provide the basis for a simple model of the dielectric constant in the different regions of the membrane-water system.

Dielectric Function Model

It is reasonable to assume that the bulk water dielectric strength is attained at least after a few water molecule layers out from the interface, and that the bulk lipid dielectric value is attained below about the ester groups. In between, in the transition region of not more than 10 Å, the proper model to use is ambiguous. This is to be expected since the dielectric constant is a macroscopic concept and has no unique meaning on the molecular scale. Nevertheless, it is judicious to proceed in describing the membrane's electrical environment with macroscopic electrostatic equations and merely use an effective dielectric constant where appropriate, as has been done in many instances to account for electrostatic properties near ions and charged electrodes. A sigmoidal functional dependence is therefore often assumed, in which the dielectric changes from a value of ~1 near the ion or interface to 80 of the bulk water in a smooth and continuous way over a distance of several to ten angstroms. This same functional shape will be used here.

Let h be the lipid headgroup region thickness, and t the half-thickness of the lipid bilayer tail region. The following dielectric function is then assumed:

$$\epsilon(z) = \epsilon_1 + \frac{\epsilon_2 - \epsilon_1}{1 + 10^{y_{\mathrm{D}}}}, \tag{9}$$

where ϵ_1 and ϵ_2 are the bulk membrane and water dielectric constants, respectively, and $10^{y_{\mathrm{D}}} = 4(t + h/2 - z)/h$. This functional dependence for y_{D} gives a dielectric transition region of width h centered at $t + h/2$. As shown in Fig. 3, observe that Eq. 9 has the required shape and takes on the proper values in the bulk aqueous and membrane phases. This equation is actually the defining relation for the model parameters t and h, whose typical membrane values are in the range $t \simeq 10$–20 Å and $h \simeq 4$–10 Å. While t is

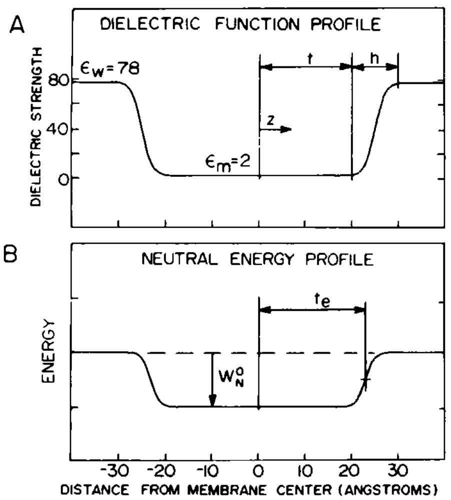

Figure 3 Dielectric and neutral energy profiles based on Eqs. 9 and 10 of the text.

thought of as the distance from the center of the bilayer to approximately the ester groups, it is actually a parameter defined by the half-thickness of the membrane for which the dielectric takes on its bulk lipid value. Similarly, h is thought of as the headgroup region thickness, but is defined by Eq. 9 as the thickness over which the dielectric constant changes.

The Born-image energy expression (Eq. 3 or 4) is written in terms of the distance x from the dielectric interface. In terms of the distance z from the bilayer center, it was pointed out above that $x = t_\epsilon - z$ where t_ϵ is the effective (electrical) bilayer half-thickness. Clearly the reasonable limits on t_ϵ are $t \lesssim t_\epsilon \lesssim t + h$, and indeed good numerical results are obtained for t_ϵ anywhere in this range. We wish to argue, however, that the best choice for the dielectric interface is at $t_\epsilon = t + h/4$, since here $\epsilon(t_\epsilon) = 10$. The reason for this is simply that the electrical energies under consideration vary inversely with the dielectric constant, and thus they are reduced dramatically unless $\epsilon(z) < 10$. The point at which $\epsilon(z) = 10$ is therefore taken as the best definition for the dielectric "interface", and this occurs at $t_\epsilon = t + h/4$.

Neutral Energy

The neutral energy contribution is essentially the free energy of transfer for moving a hydrophobic ion from the aqueous phase to the membrane, apart from electrical contributions. As mentioned in the Introduction, this is dominated by the molecule's hydrophobic energy, and therefore the discussion will focus on this aspect. Other possible contributions such as steric factors are not excluded and are considered further in the Discussion.

Neutral Energy Model

The model for the neutral energy term in the total energy function is particularly simple. It is defined as zero in the bulk aqueous phase and it takes on a constant value, $W_N{}^0$, throughout the interior of the bilayer. It remains to identify a function that provides a smooth connection between these two values over a short distance. $W_N{}^0$ is defined as the free energy of transfer for a particular hydrophobic ion in going from the aqueous to the membrane phase, apart from electrical contributions, and will typically lie in the range -4 to -8 kcal/mol, as will be thoroughly analyzed in the Discussion.

Given the above definitions, we express the neutral energy profile as follows:

$$W_N(z) = \frac{W_N^0}{1 + 10^{y_N}}, \tag{10}$$

where $y_N = -2(t + h/4 - z)/r$. This functional dependence for y_N gives a neutral energy transition region of width $2r$ (the ion size) centered at $t_\epsilon = t + h/4$. As shown in Fig. 3, observe that Eq. 10 gives a reasonable shape and takes on the proper values in the bulk aqueous and membrane phases.

RESULTS

Total Membrane Potential Model

The individual contributions to the total membrane energy profile developed in the preceding sections can now be combined into a total potential profile as given by Eq. 1. The neutral energy is given by Eq. 10 above, with the electrical contributions of Eq. 4 for the Born-image energy, Eqs. 6–8 for the dipole energy, and Eq. 9 for the dielectric function profile.

It remains to choose the most appropriate values for the model parameters. The dielectric constants in the bulk portions of the membrane and in water as discussed above are well defined by $\epsilon_1 = 2$ and $\epsilon_2 = 78$ (25°C). Also from the above discussion on the sources and magnitude of the dipole potential, it is reasonable to choose an effective dipole moment per lipid, in which case $a \simeq (60\text{–}70\ \text{Å}^2)^{1/2} \simeq 7.5\text{–}8.5$ Å (or half this per acyl chain, $a \simeq 4$ Å). Given typical lipid bilayer dimensions and the definitions of t and h in terms of the dielectric function (Eq. 9), t should lie in the range 10–20 Å, and h in the range 4–10 Å. The dipole layer should lie near the headgroup region, that is, p should range approximately between t and $t + h$ (see Table I for a summary of all parameters).

This leaves as the major parameters to be varied within reasonable values: the effective point dipole moment, u; the effective ion radius, r; and the neutral free energy of transfer between the bulk phases, $W_N{}^0$. Typical dipole moments consistent with the ester groups as the primary source, and which also give the experimentally observed dipole potentials in monolayers and bilayers, lie in the range $0-1.5$ D. Since the hydrophobic ions of primary interest here are the structural analogues TPP^+ and TPB^-, a typical potential profile for them will be considered first. Electrochemical studies and structural considerations are consistent with TPP^+ and TPB^- both having an effective radius of 4.2 Å (31), although this could be varied somewhat as a parameter to account for the approximations inherent in the Born-image energy formulation, as previously discussed. The neutral free energy of transfer values will likely fall in the range -4 to -8 kcal/mol, based on a variety of measures of the hydrophobic and neutral energy, discussed below.

Neutral Energy Contributions of TPP^+ and TPB^-

It is often convenient in theoretically treating the free energy of transfer of ionic molecules to identify distinct electrical and non- electrical contributions. Since the difficulties in doing so arise primarily from the detailed molecular interactions between the ion and nearest neighbor solvent molecules, it is expected that as ionic size increases this separation between distinct contributions becomes more valid. Thus, as for the case of TPP^+ and TPB^-, Grunwald, Baughman and Kohnstam (31) conclude "that large organic ions with low density of surface charge are solvated much as if the central charge were not there" (also see references 8, 58, and 64). An evaluation of the best estimate for $W_N{}^0$ in the total potential model rests on this observation.

From the solubility of tetraphenylmethane (TPC°) in water, 1.6×10^{-8} M (18), and in benzene, 1.4×10^{-2} M (54), a partition coefficient of $\sim 10^6$ is estimated, and this gives a free energy of transfer of about -8 kcal/mol. This is a reasonable estimate for the free energy of transfer of TPX-type molecules generally. Thus the free energies of transfer for TPC° and TPGe° from water to a variety of organic solvents of moderately high dielectric constants ($\epsilon \simeq 30$) turn out to be in the range -6 to -8 kcal/mol (42).

The Electrochemical Similarity of TPP^+ and TPB^-

A fundamental assumption in much of the analysis presented here is that TPP^+ and TPB^- are almost identical in their electrochemical properties, apart from the sign of their charge. There is substantial evidence in support of this assumption. Grunwald et al. (31) carefully studied the partial molar free energies of hydration for TPP^+, TPB^-, and TPC° in dioxane-water mixtures and first proposed the "extrathermodynamic assumption" that TPP^+ and TPB^- should have essentially the same free energies of transfer. They found good agreement between their experimental and theoretical values if the charged species were related to those of the neutral form (TPC°) by a simple Born model, a conclusion strongly supported by the work of Kim (43). From structural evidence they estimated the average unhydrated radii of TPP^+ and TPB^- to be 4.2 Å, and this value in the Born free energy of transfer expression gave good agreement with experiment. While some NMR and infrared studies question this assumption, arguing that specific solvent interactions may be important[2] (16), others point out that the similarity assumption of these molecules is consistent with hydration data obtained from alternative extra-thermodynamic assumptions, such as use of a ferrocene-ferricinium couple or an assumed liquid junction potential (58, 64).

Assessment of the Total Potential Model

The total potential model for hydrophobic ion interactions with membranes is given by Eq. 1 above, with the separate terms modeled by Eqs. 4 and 6–10, with the parameters as summarized in Table I. The parameters t, h, p, rq, ϵ_1, and ϵ_2 are either fairly well defined or their exact values within the reasonable range indicated are not very critical to the overall results. There are thus only two parameters of crucial importance: u/a^2 and $W_N{}^\circ$. By comparison, experiment places a number of constraints on the model. The model must account for the free energies of binding and translocation for both TPP^+ and TPB^-, in addition to being consistent with the individual enthalpy and entropy contributions. The best set of experimental data are taken from the results and discussion of the accompanying article (27), and are summarized here in Table II. Given this large set of experimental data, the model is well determined.

Membrane potential characteristics in the center of the bilayer are especially unambiguous. For an ion of radius 4.2 Å, the Born-image energy reaches a maximum value in the center of a 40-Å-thick bilayer of $\sim$ 17 kcal/mol, relative to the bulk aqueous phase. This figure is fairly well defined, changing by $\sim 1\%$ for a 10% change in bilayer thickness, and changing $\sim 5\%$ for a 5% change in ion radius. In addition, the profile is reasonably flat across most of the interior. Similarly, the neutral energy profile in the center of the bilayer is expected to be flat and approximately the same for both TPP^+ and TPB^-. It

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[2] It has been suggested that the interaction between otherwise identical ions of opposite sign with the water quadrupole moment can produce significant differences in hydration energies, of a much as 6 kcal/mol (4, 16). But consideration of this factor simply in addition to the energies already dealt with is inappropriate. The specific number of 6 kcal/mol refers to a theoretical model for ion solvation energies worked out by Bockris and Reddy (9); however, careful consideration of their analysis results in the following observations: (*a*) their treatment is specifically for small ions and their numerical results depend on the assumption of four waters of solvation, which is rarely the case for large ions; (*b*) their model makes use of a very specific assumed local organization of water around the central ion, and in fact this entails an additional 10 kcal/mol difference between anions and cations which is not even included in the 6 kcal/mol value; and (*c*) it has already been pointed out that taking an effective ion radius for TPP^+ and TPB^- of 4.2 Å gives good agreement with experimental free energies of transfer when incorporated into the simple Born expression (8, 31, 43). More careful calculations, in conjunction with experimental data, show the differences in free energies of transfer for molecules such as TPP^+ and TPB^- between several different solvents to be very small, typically < 1 kcal/mol (43).

TABLE II
THERMODYNAMIC VALUES FOR BINDING AND TRANSLOCATION OF TPP^+ AND TPB^- IN KCAL/MOL (25°C)

	Experimental*		Theoretical§	
	TPP^+	TPB^-	TPP^+	TPB^-
$\Delta G^{\circ\ddagger}$	20 ± 2	13.5 ± 2.5	17.8	13.2
ΔG°	−2.8 ± 0.5	−7.5 ± 0.9	−3.0	−8.6
ΔH°	+3.5 ± 0.3	−2.5 ± 1.5	+2.3	−1.8
$-T\Delta S^\circ$	−6.3 ± 0.4	−5.0 ± 1.8	−5.3	−6.8

*Experimental data for activation ($\Delta G^{\circ\ddagger}$) and binding ($\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$) are from Table II and the Discussion of reference 27; the range of values given for TPB^- include the experimental values and errors summarized there. Note that the TPP^+ data is for egg PC vesicles while that for TPB^- is for two different planar bilayer systems.

§Theoretical results are from Eqs. 1, 4, and 6–10 of the text with the following parameter values: $t = 18$ Å, $h = 8$ Å, $p = 22$ Å, $r = 4.2$ Å, $a = 8.13$ Å, $u = 0.85$ D, $q = e$, $\epsilon_1 = 2$, $\epsilon_2 = 78$, and $W_N^0 = -7.5$ kcal/mol.

follows that the energy differences in the center of the bilayer, relative to zero in the aqueous phase, must be entirely accounted for by the dipole potential. Thus the experimental free energies of activation ($\Delta G^{o\ddagger}$) and binding (ΔG°) for TPP^+ and TPB^- (Table II) determine the maximum value for the membrane potential in the center of the bilayer relative to zero in the aqueous phases: $|W_D(z = 0)| = (\Delta G^{o\ddagger}{}_+ - \Delta G^{o\ddagger}{}_- + \Delta G^\circ{}_+ - \Delta G^\circ{}_-)/2 \simeq 5.5 \pm 1.5$ kcal/mol, or 240 ± 70 mV. For a dielectric strength of 2 in the membrane this corresponds to an average dipole strength of $u \simeq 0.9 \pm 0.3$ D per 66 Å^2, which must be the range of values for the dipole potential if this model is to account for the observed effects. The magnitude of the neutral energy is also indicated by the thermodynamic data of Table II, that is, $W_N^\circ \gtrsim 5.5$ kcal/mol. Note that these experimental values are entirely consistent with those expected on the basis of general considerations already presented.

Actual binding energies and corresponding free energies of activation for translocation across the bilayer are not so simply related. The real test of the model is whether the same set of parameters that can account for the barrier heights in the center of the bilayer can also give reasonable values at the binding sites. Varying the few free parameters within the reasonable ranges given above such that the overall model conforms as closely as possible with the complete set of experimental data indeed does give good results, as shown in Fig. 4 and summarized in Table II. Comparing the theoretical and experimental results in Table II, it is apparent that the binding and translocation values for both TPP^+ and TPB^- are well accounted for by the model. Individual enthalpies and entropies of binding are also well accounted for within ~1 kcal/mol. Assuming the best-fit neutral energy of −7.5 kcal/mol is primarily entropic, and comparing this to the entropy of binding in Table II, we can also conclude that there should be no more than ~1 kcal/mol entropy contribution to the free energy of activation for TPP^+ and TPB^- transport. This has in fact been observed for hydrophobic anions within experimental accuracy (12).

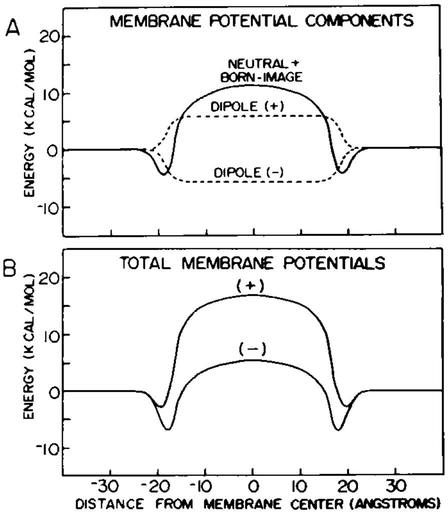

FIGURE 4 Membrane potential components and total potential profiles based on Eqs. 1, 4, and 6–10 of the text, characteristic of hydrophobic anions (–) and cations (+). (*A*) Combined neutral plus Born-image energy profile (—) together with dipole potential profiles (---). (*B*) Resulting total potential energy profiles giving a good fit to the experimental data for TPP^+ and TPB^- (Table II) with the following parameter values: $t = 18$ Å, $h = 8$ Å, $p = 24$ Å, $a = 8.13$ Å, $u = 0.85$ D, $r = 4.2$ 1V, $q = e$, $\epsilon_1 = 2$, $\epsilon_2 = 78$, and $W_N{}^\circ = -7.5$ kcal/mol.

Overall, the final potential profiles of Fig. 4 are qualitatively quite similar to those first described by Ketterer, Neumcke, and Läuger (42). Potential wells are located near the membrane-water interfaces, toward the membrane side, and broad energy barriers are located in the middle of the membrane. Two important new aspects are, however, also revealed. The dipole potential is now explicitly included in the total energy profiles, and this results in substantial energy difference between cations and anions in the center of the bilayer. In addition, the introduction of a more realistic dipole potential profile near the interfaces gives rise to differences in the location and energy of the binding sites. Thus the potential well for cations is more shallow and the translocation barrier is higher than that for structurally similar anions.

Further Applications of the Potential Model

While this model was developed specifically for hydrophobic ions, it should in fact be quite general. Thus if the parameter values giving the best fit for the TPP^+ and TPB^- data are now taken as characteristics of lipid bilayers generally, several other consequences follow from the model. These include the effects of ion size, changes in the dielectric constants of either region, alterations in the location and strength of the dipole sources, and the quantitative effects of agents that modify the dipole potential.

The potential profiles for smaller ions are one such example. Using the best fit parameters from above, and changing only the effective ion radius and assuming no neutral energy contribution, the membrane potential profiles for 3 Å ions is obtained as shown in Fig. 5 *a*. Two prominent features thus become apparent. While the barriers in the center of the bilayer are quite substantial, the anion barrier is nevertheless significantly lower than that for the cation by at least 10 kcal/mol. Since translocation rates vary exponentially with the free energy of activation, this suggests that such anions would be much more membrane permeable than cations. The other resulting feature of interest is the possible existence of slight binding pockets (~1 kcal/mol) for anions near the membrane surface, a unique result that only arises if the dipole potential is felt by the ion before the strong repulsive Born energy becomes important. While these findings may relate qualitatively to the lipid bilayer permeability of small ions such as Cl^- or Br^-, they are particularly attractive in explaining the properties of "chaotropic" ions such as thiocyanate, iodide-iodine complex, perchlorate and nitrate, all of which are fairly large ions with significant charge delocalization and are known to readily permeate membranes. Slight binding pockets near the interface, as in Fig. 5 *a*, have in fact been observed experimentally (53). A more detailed analysis of these ions would require consideration of any neutral energy term and modifications of the Born-image energy for molecules of different shape or charge distribution.

Modifications of the membrane dipole potential can also be analyzed. For example, small polar groups with large dipole moments—e.g., CF_3 groups or nitroxides, which have dipole moments of ~2 D—can be introduced along the fatty acid chains. Such alterations can be modeled by introducing a second dipole layer of the same functional form as given by Eqs. 6–8 but at a different location, p'. Thus in Fig. 5 *b* is shown the result of introducing a 1 D effective dipole moment per lipid molecule placed half way along the fatty acid chain, where the orientation is the same as that of the intrinsic membrane dipoles. The net effect is to significantly increase the cation barrier while reducing the anion barrier throughout the bulk of the membrane. Choices of location, strength and orientation can give rise to a number of interesting alterations in the internal membrane potential. The possibility of "designing" the internal membrane potential for specific purposes is possible experimentally (44; and see references 24 and 40 for reviews), and now can also be treated theoretically.

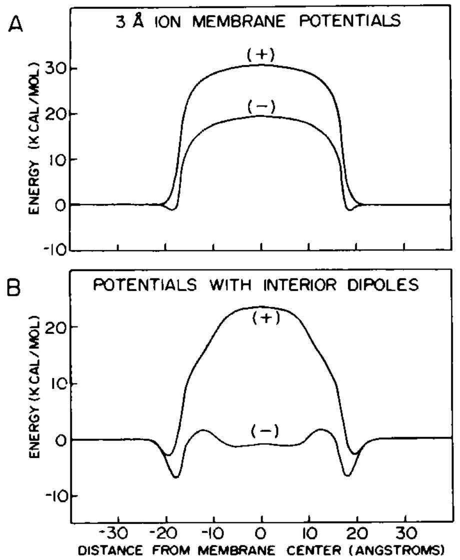

FIGURE 5 Total membrane potential profiles (*A*) for small (3 Å) ions assuming no neutral energy contribution (notice possible binding pockets for anions), and (*B*) for an additional dipole layer located 10 Å from the bilayer center and with point dipoles of strength 1 D. Parameters are otherwise as in Fig. 4.

DISCUSSION

The total membrane potential model presented here can, with a single set of reasonable parameters, successfully account not only for the binding and translocation properties of both hydrophobic cations and anions, but also for individual enthalpic and entropic contributions. We believe this agreement strongly supports the description of hydrophobic ion interactions with membranes represented by Eq. 1 in terms of four principle components: the Born, image and dipole electrical energies, and the neutral energy. While other terms such as steric factors and specific local electrostatic interactions may also play a role, they appear to be only of secondary importance.

The specific parameter values used in the model that give good agreement with experimental data are all within the fairly narrow range of values expected based on general theoretical and experimental considerations, and discrepancies that arise amount to no more than ~ 1 kcal/mol. Since much of the thermodynamic data can be accounted for with only a few free parameters, and since the results are rather insensitive to the detailed choices of t, h, p, r, ϵ_1, and ϵ_2, the model is well characterized. For example, using the best overall parameters to fit the TPP^+ and TPB^- data (Fig. 4 and Table II), and varying r and ϵ_1 by 5% has no significant effect on the location or strength of the binding sites and affects the maximum barrier height by no more than 1 kcal/mol. The actual critical parameters in the model are u/a^2 and $W_N{}^\circ$. To fit the TPP^+ and TPB^- data adequately, reasonably good results were obtained here for $u \simeq 0.9 \pm 0.1$ D (for $a^2 = 66$ Å^2), and $W_N{}^\circ \simeq -7.0 \pm 1.0$ kcal/mol. This range for u corresponds to ~5.5 ± 0.5 kcal/mol dipole energy at maximum, or ~240 ± 20 mV, positive inside, dipole potential. Values more than ~1 kcal/mol (40 mV) outside this range would indicate the existence of other contributions to the total potential profile not specifically treated.

For general applications of this model other considerations would be important. These would include modifications of the Born energy as for ion shape or charge distribution, higher multipole moments in the Born-image and dipole expressions, specific local electrical interactions with the lipid groups, and steric factors. Formally these other energies would pose no real problems in terms of the theoretical model. An additional term representing an extra attractive or repulsive energy with a suitable localized shape could easily be added. It may very well be that hydrophobic anions such as TPB$^-$, DPA$^-$, and PCB$^-$ have a favorable electrostatic interaction with quaternary ammonium groups, and that this may be a contributing factor in explaining why these molecules bind so strongly. Such an energy term would shift the anion binding site out toward the headgroup, with the dipole potential primarily providing the translocation differences observed. Special cases of molecules with large dipole moments or large polarizabilities may be of some interest and have already received some attention (11, 24).

Several important general implications for membrane energetics also follow from the results here. One set of implications relate to the general nature of hydrophobic ion interactions with membranes. The results of Fig. 4 and Table II, and the accompanying discussion, show that for otherwise identical ions, the dipole potential accounts for the differences in the total energy in the center of the membrane, as well as for significant differences in binding. The potential minima, as shown in the figure, account for the much stronger anion binding over that for the cation, and it locates the binding site below the headgroup region in the vicinity of the ester groups. The profiles also suggest that the cation binds slightly further out than the anion, although the difference of a few angstroms is only of marginal significance given the spatial resolution of the model.

Another set of implications relate to the basic electrostatic structure of lipid bilayers. The data and the model are all consistent with lipid bilayers possessing a dipole potential of several hundred millivolts. This feature should be of great importance to the energetics of a wide range of membrane processes, such as the interaction of other charged molecules with bilayers, and the structural and functional properties of membrane proteins generally.

APPENDIX A

Eq. 3 of the text is the general theoretical expression for the combined Born and image energies for a charge within a thin dielectric slab sandwiched between two bulk dielectrics. For conceptual and computational simplification it is useful to consider several limiting cases of this general expression.

In the limit of perfect conductors, $\epsilon_1/\epsilon_2 \rightarrow 0$ ($\alpha \rightarrow 1$)—a good approximation for a membrane surrounded by aqueous phases—the energy expression can be put into a more concise form. Introducing the notation $\bar{\psi}(y) = \psi(y) + \psi(1 - y)$, where $\psi(y)$ is the Psi (or, Digamma) function (1, 30), the Born-image energy profile can be written:

$$W_{B-I}(x) = \left[\frac{1}{4\pi\epsilon_0}\right]\frac{q^2}{4\epsilon_1}\left[\frac{1}{r} + \frac{1}{d}\bar{\psi}\left(\frac{x}{d}\right) - \frac{1}{d}\bar{\psi}\left(\frac{r}{d}\right)\right]. \qquad \text{(A1)}$$

A more convenient power series form can be obtained upon noting that:

$$\bar{\psi}(y) = -2\gamma - 1/y - 2\sum_{n=1}^{\infty}\zeta(2n + 1)y^{2n}$$

where

$$\zeta(s) = \sum_{n=1}^{\infty} n^{-s}$$

is the Riemann zeta function and γ is Euler's constant. Thus,

$$W_{B-I}(x) = \left[\frac{1}{4\pi\epsilon_0}\right]\frac{q^2}{2\epsilon_1} \cdot \left\{\frac{1}{r} - \frac{1}{2x} - \frac{1}{d}\sum_{n=1}^{\infty}\zeta(2n + 1)\left[\left(\frac{x}{d}\right)^{2n} - \left(\frac{r}{d}\right)^{2n}\right]\right\}. \qquad \text{(A2)}$$

For normal conditions of interest ($r \lesssim 4$ Å, $d \simeq 40$ Å, $r \leq x \leq d/2$), the first term of the sum in Eq. A2 differs numerically from the full expression by not more than ~4% (it is good to better than 1% in the conductor limit). Taking only the first term then gives Eq. 4 in the text as the simplified expression for the Born-image energy.

APPENDIX B

The Born-image energy expressions (Eqs. 3 and 4 of the text) become negative for values of $x \lesssim r/2$, under which conditions, as discussed in the text, it is reasonable to set the Born-image energy equal to zero. But such a procedure introduces an abrupt change in the slope at the transition point. To obviate this problem, a "tail" function is defined for $x < r$ which has the following theoretical form:

$$W_{B-I}(x) = \left[\frac{1}{4\pi\epsilon_0}\right]\frac{q^2}{2\epsilon_1 r}\frac{1}{1 + 10^{y_T}} \qquad (x < r),$$

where $y_T = -2(x - r)/r$. This function gives the edge of the Born-image energy a smooth transition "tail" of width $2r$ centered at $x = r$ and was used in the total potential model for this purpose.

This work was supported by National Institutes of Health grant EY05216 and a Vision Research Center grant EY00331 to the Jules Stein Eye Institute, and the Jules Stein Professorship Endowment.

Received for publication 18 April 1985 and in final form 3 September 1985.

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