Hydrophobic Ion Hydration and the Magnitude of the Dipole Potential

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ABSTRACT   The magnitude of the dipole potential of lipid membranes is often estimated from the difference in conductance between the hydrophobic ions, tetraphenylborate, and tetraphenylarsonium or tetraphenylphosphonium. The calculation is based on the tetraphenylarsonium-tetraphenylborate hypothesis that the magnitude of the hydration energies of the anions and cations are equal (i.e., charge independent), so that their different rates of transport across the membrane are solely due to differential interactions with the membrane phase. Here we investigate the validity of this assumption by quantum mechanical calculations of the hydration energies. Tetraphenylborate ($\Delta G^{hydr} = -168$ kJ mol^{-1}) was found to have a significantly stronger interaction with water than either tetraphenylarsonium ($\Delta G^{hydr} = -145$ kJ mol^{-1}) or tetraphenylphosphonium ($\Delta G^{hydr} = -157$ kJ mol^{-1}). Taking these differences into account, literature conductance data were recalculated to yield values of the dipole potential 57 to 119 mV more positive in the membrane interior than previous estimates. This may partly account for the discrepancy of at least 100 mV generally observed between dipole potential values calculated from lipid monolayers and those determined on bilayers.

INTRODUCTION

The extrathermodynamic TATB (tetraphenylarsonium tetraphenylborate) hypothesis, i.e., that the structurally very similar hydrophobic ions tetraphenylarsonium (TPA$^+$) and tetraphenylborate (TPB$^-$) (Fig. 1) have identical interaction energies with solvating water molecules, has found wide application in physical chemistry and biophysics. In physical chemistry it is often used as a basis for the calculation of thermodynamic properties of isolated ionic species, which are experimentally not directly accessible, e.g., free energies of hydration and free energies of transfer between different solvents (Grunwald et al., 1960; Choux and Benoit, 1969; Cox and Parker, 1973; Fawcett, 1993; Benko et al., 1996). In membrane biophysics, the different degrees of interaction of TPB$^-$, TPA$^+$, and TPP$^+$ with lipid membranes has been used to calculate the electrical dipole potential within the membrane interface with the TATB hypothesis as an underlying assumption.

A version of the TATB hypothesis was first proposed by Grunwald et al. (1960), who used it to calculate the rate of change of the standard partial molar free energy with changing mole fraction of water for a variety of single anions and cations. They reasoned that the symmetrically arranged phenyl residues around the central charged atom should act as an insulating layer, protecting the charge from interaction with surrounding solvent molecules. If the thickness of the insulating layer were sufficiently large with the charge remaining buried at the center and very low surface charge

density, the solvation should closely resemble that of an uncharged molecule of equal size and structure (e.g., tetraphenylmethane) and the sign of the charge should become irrelevant. According to Grunwald et al. (1960) this limiting situation should already be reached by TPB$^-$ and TPP$^+$ with average ionic radii of 4.2 Å.

A major reason for the importance of hydrophobic ions in membrane biophysics lies in the fact that the investigation of their conductance across black lipid membranes led to the discovery of the membrane dipole potential (Liberman and Topaly, 1969). The motive for their studies was to use hydrophobic ions as model systems for the carrier mechanism of ion transport. Surprisingly, however, they discovered that the permeability of the membrane for TPB$^-$ was approximately 10^5 greater than that of TPP$^+$. To explain this difference in behavior they hypothesized that the interior of the membrane must initially be positively charged. Haydon and coworkers (Haydon and Myers, 1973; Hladky and Haydon, 1973) later recognized that the positive charge within the membrane must arise from oriented molecular dipoles in the membrane surface and coined the term "dipole potential."

The absolute magnitude of the dipole potential has been estimated by a number of groups (Andersen and Fuchs, 1975; Pickar and Benz, 1978; Flewelling and Hubbell, 1986; Gawrisch et al., 1992; Franklin and Cafiso, 1993)
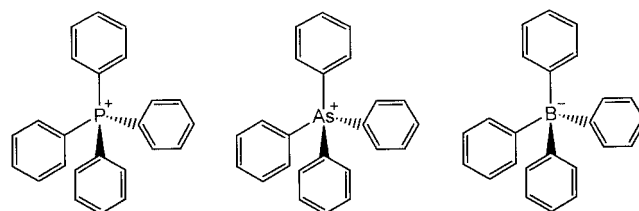
FIGURE 1   Structures of the hydrophobic ions, tetraphenylphosphonium (TPP$^+$), tetraphenylarsonium (TPA$^+$), and tetraphenylborate (TPB$^-$).

from the magnitude of the relative conductivities of membranes for hydrophobic anions and cations as first observed by Liberman and Topaly (1969). A high membrane conductance requires the hydrophobic ions to be able to move from one side of the membrane to the other. The membrane itself can, therefore, be seen as an activation energy barrier for ion diffusion. Thus, we can define a rate constant for ion diffusion, k, which is related to the free energy of transfer of an ion from the aqueous phase into the membrane, $\Delta G^{\#}$, simply by the Arrhenius equation. The free energy of transfer of a hydrophobic ion into the membrane is actually made up of a number of individual free energy terms (Ketterer et al., 1971; Flewelling and Hubbell, 1986b; Benz, 1988):

$$\Delta G^{\#} = \Delta G_{B}^{\#} + \Delta G_{I}^{\#} + \Delta G_{D}^{\#} + \Delta G_{N}^{\#} \qquad (1)$$

The individual contributions relate to the Born energy difference ($\Delta G_{B}^{\#}$), due to the change in dielectric constant between water and the membrane (Born, 1920), the image energy contribution ($\Delta G_{I}^{\#}$), due to the polarization of water molecules adjacent to the aqueous solution-membrane interface (Silver, 1985; Neumcke and Läuger, 1969), the dipole energy difference ($\Delta G_{D}^{\#}$), due to the interaction of the hydrophobic ion with the intrinsic dipole potential of the membrane (Flewelling and Hubbell, 1986), and the neutral energy difference ($\Delta G_{N}^{\#}$), which takes into account all interactions which a hypothetical neutral particle (generated by discharging the ion) would have with the aqueous phase and the membrane, e.g., van der Waals and steric interactions. Now it is important to note that all of the energy terms except for the dipole energy contribution are independent of the sign of the charge of the ion. Eq. 1 can, therefore, be simplified to:

$$\Delta G^{\#} = \Delta G_{o}^{\#} + zF\Psi_{d} \qquad (2)$$

in which $\Delta G_{o}^{\#}$ includes all energy terms except for the interaction of the hydrophobic ion with the dipole potential, ψ_{d}, in which z and F are the valence of the ion and Faraday's constant, respectively. Now, if it is possible to find a hydrophobic anion and a hydrophobic cation whose values of $\Delta G_{o}^{\#}$ are identical, then the difference in their rate constants for ion diffusion would be solely due to the value of the dipole potential. Under these conditions, subtracting the relevant forms of the Arrhenius equation for an anion and a cation from one another and rearranging yields:

$$\Psi_{d} = \frac{RT}{2F} \ln \frac{k_{-}}{k_{+}} \qquad (3)$$

in which k_{-} and k_{+} are the rate constants for the transfer of the anion and the cation across the membrane, respectively, R is the ideal gas constant, and T is the absolute temperature.

Because of their very similar sizes and chemical structures and their supposedly identical hydration energies (TATB hypothesis), the two ions normally chosen for mea-

surements of the dipole potential of lipid bilayers are TPB^{-} and TPA^{+} or TPP^{+}. Rather than use rate constants as shown in Eq. 3, it is, however, more usual to use the specific conductances, g, in which case k_{-} and k_{+} must simply be replaced in Eq. 3 by g_{-} and g_{+}. The application of Eq. 3 to find an absolute value of the dipole potential requires that the values of $\Delta G_{o}^{\#}$ for TPB^{-} and TPA^{+} or TPP^{+} are equal. As pointed out by several authors (Andersen and Fuchs, 1975; Pickar and Benz, 1978; Gawrisch et al., 1992), however, this assumption may be questionable. Even though the hydrophobic anions, TPB^{-} and TPA^{+}, have almost identical sizes, their values of $\Delta G_{o}^{\#}$ could differ, in particular, if their free energies of hydration are different, i.e., the TATB hypothesis is not valid.

In fact, experimental evidence has been reported, which suggests that the interactions of TPB^{-} with water are significantly different from TPA^{+} and TPP^{+}. Different interactions of solvent protons with the phenyl rings of TPB^{-} and both TPA^{+} and TPP^{+} have been reported by Coetzee and Sharpe (1971). Differences in the interactions of water molecules with TPB^{-} and either TPP^{+} or TPA^{+} have also been reported by Józwiak and Taniewska-Osinska (1994), Stangret and Kamienska-Piotrowicz (1997), and Symons (1999). From their measurements, Stangret and Kamienska-Piotrowicz (1997) estimated that the interaction of water with TPB^{-} could be up to 16 kJ mol^{-1} stronger than that of the interaction with TPP^{+}.

Over the last decade major advances have been made in the development of methods for quantum mechanical calculations on complex molecules (Frisch et al., 1998; Barone and Cossi, 1998). The time is, therefore, ripe to carry out a theoretical analysis of the validity of the TATB hypothesis. The goals of the present paper are twofold: 1) calculate theoretically the free energies of hydration of TPB^{-}, TPA^{+}, and TPP^{+} and 2) based on the calculated hydration energies, redetermine the magnitude of the dipole potential.

According to the quantum mechanical calculations it will be shown that previous estimates of the dipole potential of lipid bilayers are likely to be significantly underestimated.

THEORY

If one does not accept the TATB hypothesis, one can write a modified version of Eq. 2 for the free energy of transfer of a hydrophobic ion into the membrane:

$$\Delta G^{\#} = \Delta G_{o}^{\#} - \Delta G_{hyd} + zF\Psi_{d} \qquad (4)$$

ΔG_{hyd} represents the free energy of hydration of the ion and $\Delta G_{o}^{\#}$ now includes all energy terms except for the hydration energy and the interaction of the ion with the dipole poten-

tial. The corresponding form of the Arrhenius equation is now:

$$\ln k = -\frac{\Delta G_o^{\#} - \Delta G_{hyd} + zF\Psi_d}{RT} \quad (5)$$

Subtracting the relevant forms of Eq. 5 for hydrophobic anions and cations from one another and rearranging now yields:

$$\Psi_d = \frac{RT}{2F} \ln \frac{k_-}{k_+} + \frac{\Delta G_{hyd}^+ - \Delta G_{hyd}^-}{2F} \quad (6)$$

If one measures the specific conductances of TPB^- and TPA^+, the relevant expression is:

$$\Psi_d = \frac{RT}{2F} \ln \frac{g_{TPB^-}}{g_{TPA^+}} + \frac{\Delta G_{hyd}^{TPA^+} - \Delta G_{hyd}^{TPB^-}}{2F} \quad (7)$$

If the free energies of hydration of TPA^+, TPP^+, and TPB^- are known and the rate constants of transfer or specific conductances of the ions have been measured, the application of Eq. 6 or 7 allows the absolute magnitude of the dipole potential of the membrane to be directly estimated.

Implicit in Eqs. 6 and 7 is the assumption that the ions TPA^+, TPP^+, and TPB^- undergo complete dehydration on binding to the membrane and do not carry any water with them through the bilayer. Although there is as yet no direct evidence supporting this assumption, indirect evidence exists suggesting that it is likely to be the case. For a range of anions it has recently been found (Clarke and Lüpfert, 1999) that those with low hydration energies interact most strongly with phospholipid membranes. This strongly suggests, therefore, that the energetics of anion dehydration play a dominant role in determining the strength of anion-membrane binding.

COMPUTATIONAL METHODS

The calculations reported in this paper were performed using a range of different methods. All equilibrium structures were determined by application of density functional theory using the B3LYP functional (Lee et al., 1988; Miehlich et al., 1989; Becke, 1993) in conjunction with the 6-31G and 6-31G(d) basis sets (Ditchfield et al., 1971; Hehre et al., 1972; Hariharan and Pople, 1973, 1974; Gordon, 1980). The density functional theory structures, obtained at the B3LYP/6-31G level of theory, were verified as local minima on the potential energy surface by frequency analysis. The partial charges of individual atoms were calculated using the Mulliken method of population analysis (Mulliken, 1962).

Solvation effects were computed by application of the conductor-like screening model (COSMO) (Klamt and Schüürmann, 1993; Klamt, 1995; Klamt and Jonas, 1996; Barone and Cossi, 1998) with water ($\epsilon = 78.39$) as the solvent. COSMO is basically a dielectric continuum model, which approximates the dielectric continuum by a scaled conductor. It is a fast, reliable, and widely accepted method. The deviations between COSMO and rigorous dielectric continuum methods are negligible in strong dielectrics such as water. A completely reliable estimation of the accuracy of the calculated hydration energies of hydrophobic ions is unfortunately not possible because no experimental values are available for comparison. Barone and Cossi (1998), however, calculated the hydration energies using the COSMO model for 19 neutral molecules. Using the B3LYP functional they found an average deviation of the calculated and experimental values of 21%. Of the 19 molecules for which they performed calculations, all except one gave a calculated hydration energy, which was lower than that of the experimental value by at least 6%. COSMO, thus, appears to underestimate slightly the true value of the hydration energy. Because of this systematic deviation, the error in the difference in hydration energies of two related organic compounds may be less than that of their individual absolute values. For the calculations presented here, the differences in the hydration energies of TPB^- and TPP^+ or TPA^+ are conservatively estimated to have an accuracy of $\pm 20\%$.

The density functional theory and COSMO computations were performed using the Gaussian 98 programs. All computations were performed on DEC α600/5/333 and COMPAQ XP1000/500 workstations of the theoretical chemistry group at the University of Sydney.

RESULTS

Hydration energies

The free energies of hydration of the ions TPB^-, TPP^+, and TPA^+ were theoretically calculated using the procedure described under Methods. The values obtained for TPB^-, TPP^+, and TPA^+ using the 6-31G basis set were -166 kJ mol^{-1}, -154 kJ mol^{-1}, and -145 kJ mol^{-1}, respectively. Using the more expanded 6-31G(d) basis set similar results were obtained: -168 kJ mol^{-1}, -157 kJ mol^{-1}, and -145 kJ mol^{-1} for TPB^-, TPP^+, and TPA^+, respectively. The interaction of TPB^- with water is, thus, found to be significantly stronger than both TPP^+ and TPA^+. The differences in the hydration energies between TPB^- and TPP^+ are 12 kJ mol^{-1} and 11 kJ mol^{-1} for the 6-31G and 6-31G(d) basis sets, respectively. The differences between TPB^- and TPA^+ are 21 kJ mol^{-1} and 23 kJ mol^{-1}, for the 6-31G and 6-31G(d) basis sets, respectively.

For comparison, calculations were also performed on the uncharged analogue, tetraphenylmethane. For this molecule the hydration energy was found to be -16 kJ mol^{-1} using the 6-31G basis set, i.e., 129 to 150 kJ mol^{-1} weaker than the solvation of TPB^-, TPP^+, and TPA^+. This result indicates, as initially pointed out by Grunwald et al. (1960), that the phenyl groups are in fact not perfect insulators and that significant interaction between the charges of the hydrophobic ions and the surrounding water molecules does actually occur.

The differences in the hydration energies of TPB^- and TPP^+ are in qualitative agreement with the experimental findings of Stangret and Kamienska-Piotrowicz (1997), who found from infra-red spectroscopic measurements that the difference in hydrogen-bond energy of water surrounding TPP^+ and TPB^- ions is ~ 16 kJ mol^{-1} of ions. They found that the interaction of TPB^- with water was significantly stronger than that of TPP^+, and they attributed this effect to a higher degree of polarizability of TPB^-, which would be expected to strengthen van der Waals interactions with the surrounding water molecules.

The calculations presented here are also qualitatively consistent with the molecular dynamics study of Schurham-

mer and Wipff (1999, 2000), who found using different models that TPB$^-$ was consistently better hydrated than TPA$^+$ by ~76 to 185 kJ mol^{-1}. They attributed the difference to specific OH-π bridging interactions between water and the phenyl rings in TPB$^-$ and to an "electrostatic preorganization", i.e., in the hypothetical neutral TPB^0 and TPA^0 species the central atom is positive and thus predisposed to negative charging. Using a Langevin dipole model of the solvent, Luzhkov and Warshel (1992) also calculated TPB$^-$ to be more strongly hydrated than TPP$^+$, by approximately 28 kJ mol^{-1}. The major reason for this difference they attributed to greater negative charge delocalization in TPB$^-$ onto the phenyl rings and hence greater interaction with the surrounding solvent. In contrast, the positive charge of TPP$^+$ they found to be more localized on the central atom, which is sterically shielded from the solvent. Finally, using a model based on electrostatic theory, Marcus (1991), furthermore, calculated that the hydration energy of TPB$^-$ was 110 kJ mol^{-1} more negative than that of TPA$^+$.

Although the absolute values of the free energies of hydration and the differences between TPB$^-$, TPP$^+$, and TPA$^+$ reported here and elsewhere vary considerably, there appears to be general agreement that TPB$^-$ is significantly more strongly hydrated than TPP$^+$ or TPA$^+$.

Volumes and surface areas

One possibility to account for the differences in the hydration energies of the ions might be varying sizes. According to the Born theory of hydration of spherical ions in a homogeneous dielectric medium (Born, 1920), the hydration energy is inversely proportional to the radius of the ion. Because the sizes of the central atoms decrease in the order As > P > B, one would expect the strongest hydration energy for TPB$^-$.

For the calculation with the 6-31(d) basis set, the volumes and surface areas for TPAs$^+$, TPP$^+$, and TPB$^-$ determined are given in Table 1. Also listed are average radii of the ions, assuming spherical geometry, calculated from the volume and surface area data, respectively. The ratios of $1/r_{av}^V$ for TPAs$^+$, TPP$^+$, and TPB$^-$ correspond to 1:1.008:1.017 for the three ions. The ratios of $1/r_{av}^A$ correspond to 1:1.001: 1.022. For comparison, the ratios of the hydration energies are 1:1.080:1.159. Thus, the calculated hydration energy of TPB$^-$ is 15.9% greater than that of TPAs$^+$, whereas the calculated values of $1/r_{av}^V$ and $1/r_{av}^A$ are only 1.7 and 2.2% greater, respectively. It, therefore, appears that although the size of the ions could explain a small part of the greater hydration energy of TPB$^-$, it cannot be the only cause for its higher value. Presumably differences in charge distribution must also be playing a role, as proposed by Luzhkov and Warshel (1992).

One further point worth noting from the calculations is that for each ion the value of r_{av}^A is always significantly higher than that of r_{av}^V. This indicates that the ions are

TABLE 1 Hydration energies, ΔG, volumes, V, surface areas, A, and average radii, r_{av}^V and r_{av}^A of tetraphenylborate (TPB$^-$), tetraphenylarsonium (TPA$^+$), and tetraphenylphosphonium (TPP$^+$) calculated using the B3LYP density functional in conjunction with the 6-31G(d) basis set and the conductor like screening model (COSMO) r_{av}^V and r_{av}^A refer to the average radii calculated from the volume and area data, respectively

Ion	ΔG (kJ mol^{-1})*	V (Å^3)	A (Å^2)	r_{av}^V (Å)	r_{av}^A (Å)
TPB$^-$	−168	405.5	359.4	4.59	5.35
TPP$^+$	−157	415.6	374.5	4.63	5.46
TPAs$^+$	−145	426.0	375.4	4.67	5.47

*Accuracy of the hydration energies is estimated to be ±21% (see Computational Methods).

certainly not spherical, i.e., the surface area to volume ratio is always higher than expected for a sphere. This can, in fact, also easily be seen by constructing space-filling models of the ions. There are significant clefts between the phenyl rings into which the dielectric continuum (i.e., the solvent water) can penetrate.

Charge distribution

The charges of the individual atoms of TPB$^-$, TPA$^+$, and TPP$^+$ are given in Table 2. If one concentrates on the central atom, it is obvious that the negative charge of TPB$^-$ is not located on the central boron, which is clearly positively charged, but is instead delocalized over the carbons of the phenyl rings. The total charge on the four phenyl rings of TPB$^-$ is calculated to be −1.132. In contrast, the central P and As atoms of TPA$^+$ and TPP$^+$ both have charges significantly greater than zero, indicating that in their case the positive charge is more localized on the central atom. The total charges on the phenyl rings of TPA$^+$ and TPP$^+$ are +0.527 and +0.273, respectively, i.e., both much less than the absolute magnitude of the charge on the phenyl rings of TPB$^-$. The charges on the central atoms of TPP$^+$ and TPA$^+$ would, furthermore, be sterically hindered in their interaction with the solvent by the surrounding phenyl rings.

The significant charge delocalization of TPB$^-$ can be understood on the basis of the different electronegativities of the boron and carbon atoms. On the Pauling scale, boron has an electronegativity of 2.04, whereas carbon has a value of 2.55 (Aylward and Findlay, 1998). The electrons would, thus, be attracted by the more electronegative atom, i.e., carbon, resulting in movement of the net negative charge away from the central atom onto the phenyl rings. In the case of TPP$^+$ and TPA$^+$, both phosphorus (2.19) and arsenic (2.18) also have lower electronegativities than carbon. Movement of electrons toward the carbons of the phenyl rings would be expected in these cases as well. However, because the overall charges on TPP$^+$ and TPA$^+$ are +1, the

TABLE 2 Atomic charges (units of electronic charge) of hydrated tetraphenylborate (TPB$^-$), tetraphenylarsonium (TPA$^+$), and tetraphenylphosphonium (TPP$^+$) calculated from Mulliken population analysis using the B3LYP density functional in conjunction with the 6-31G(d) basis set

Atom*	Charges (e.u.)		
	TPB$^-$	TPA$^+$	TPP$^+$
A	0.133	0.473	0.728
C1	0.067	−0.010	−0.110
C2	−0.246	−0.182	−0.170
C3	−0.162	−0.153	−0.150
C4	−0.184	−0.141	−0.143
H2	0.114	0.192	0.199
H3	0.139	0.190	0.188
H4	0.144	0.188	0.188

*The numbering system of the atoms is shown in Fig. 2.

TABLE 3 Correction of dipole potential values, ψ_d, due to the differences in hydration energies of tetraphenylborate (TPB$^-$), tetraphenylarsonium (TPA$^+$), and tetraphenylphosphonium (TPP$^+$)

Lipid	Conductance ratio*	ψ_d (lit.) (mV)	Ref. †	ψ_d (corr.) (mV)
Bacterial PE	3×10^6	190	1	309
Glycerylmonooleate	(4×10^3)	108	2	165
Dioloeyl-PC	3.2×10^7	224	2	343
1-Oleoyl-2-stearoyl-PC	3.9×10^6	197	2	316
1-O-oleyl-2-O-palmityl-PC	4.7×10^3	110	2	229
Dioeloyl-PE	1.6×10^7	215	2	334
Di-O-oleyl-PE	9.3×10^3	119	2	238
Dipalmitoyl-PC	1.2×10^7	227	3	346
Dihexadecyl-PC	2.5×10^3	109	3	228

*The conductance ratios have been calculated by dividing the conductance of TPB$^-$ by that of TPA$^+$ (except for glycerylmonooleate where TPP$^+$ was used instead of TPA$^+$).
†The reference numbers listed: 1, Andersen and Fuchs (1975); 2, Pickar and Benz (1978); 3, Gawrisch et al. (1992).

electron movement results in the localization of the net positive charge of the ions on the central atom.

The stronger hydration energy of TPB$^-$ in comparison with TPP$^+$ and TPA$^+$ can, thus, be attributed to different charge distributions. The delocalization of the negative charge of TPB$^-$ onto the phenyl rings would allow a closer contact between the ion charge and the surrounding solvent. This would result in a greater image energy contribution to the hydration energy due to the polarization of the surrounding water molecules.

An additional steric effect was discussed by Luzhkov and Warshel (1992) who considered the orientation of the water molecules around the ions. In the case of an anion the water molecules must be orientated with their hydrogens pointing toward the ion, whereas for a cation the water molecule orientation is reversed. They found that this additional effect resulted in a further stabilization of TPB$^-$ relative to TPP$^+$. This effect is, however, not included in the present calculations, because the solvent is considered here as a dielectric continuum. It is, therefore, possible that the hydration energy of TPB$^-$ relative to TPP$^+$ and TPA$^+$ may even be slightly greater than that given in Table 3.

Dipole potential calculation

Now that theoretical values of the free energies of hydration of TPB$^-$, TPP$^+$, and TPA$^+$ have been determined, these values can be used to correct dipole potential values already reported in the literature (Andersen and Fuchs, 1975; Pickar and Benz, 1978; Gawrisch et al., 1992) where differences in hydration were not previously taken into account. For this purpose we apply Eq. 7 using the following calculated values (using the 6-31G(d) basis set): $\Delta G_{\mathrm{hyd}}^{\mathrm{TPA}^+} = -145$ kJ mol^{-1}, $\Delta G_{\mathrm{hyd}}^{\mathrm{TPP}^+} = -157$ kJ mol^{-1}, and $\Delta G_{\mathrm{hyd}}^{\mathrm{TPB}^-} = -168$ kJ mol^{-1}. The previously calculated values of the dipole potential plus the values corrected for hydration are given in Table 3. The correction amounts to an increase in the actual value of the dipole potential of 119 ($\pm$24) mV in the case of measurements where the conductivities of TPA$^+$ and TPB$^-$ have been compared and an increase of 57 ($\pm$11) mV in the case of measurements comparing TPP$^+$ and TPB$^-$.

DISCUSSION

The membrane dipole potential is currently of great interest in the field of membrane biophysics because of the possibility that it may affect the conformation of membrane proteins and thus be involved in numerous membrane-related physiological processes. The electric field produced by the dipole potential in the membrane interface is extremely large, i.e., $10^8 - 10^9$ V m^{-1} (Brockman 1994; Cafiso, 1995; Clarke, 2001), which is significantly larger than that caused by a typical total membrane potential (e.g., a 100-mV membrane potential produces an electric field stength of $\sim 2.5 \times 10^7$ V m^{-1}). It is well known from electrophysiological studies that the opening and closing of ion channels can be controlled by the membrane potential (voltage-gated ion channels). Unless it is somehow electri-
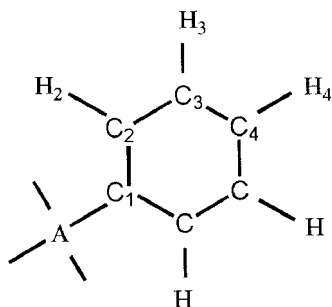

FIGURE 2 Numbering system of the individual atoms of the phenyl rings of tetraphenylphosphonium, tetraphenylarsonium, and tetraphenylborate. The central atom (i.e., P, As, or B) is designated by the symbol A.

cally shielded by oppositely charged protein residues, it is highly likely, therefore, that the dipole potential has a major effect on the conformation and kinetics of ion-transporting membrane proteins. In fact a number of examples of effects of the dipole potential on membrane-related processes have recently been reported. Nachliel et al. (1996) proposed a major effect of the dipole potential on the ion transport kinetics of the ionophore monensin. Cladera and O'Shea (1998) have suggested that the dipole potential affects the membrane insertion and folding of an amphiphilic peptide. Major effects of the dipole potential on the kinetics of ion transport through the gramicidin channel have recently been reported by both Rokitskaya et al. (1997) and Busath et al. (1998).

Because of its likely significance in physiological processes of ion transport, it is important to establish the absolute magnitude of the dipole potential. Previous estimates of the dipole potential of lipid bilayers have been based on the relative conductances of TPB^- and either TPA^+ or TPP^+ across the membrane. Either implicit or explicitly stated in the calculation of these values was, however, the assumption that the free energies of hydration of each of these ions are equal. Flewelling and Hubbell (1986), for example, assumed an equal neutral energy contribution to the transfer of TPP^+ and TPB^- into the membrane and fitted their data using a value of -29 kJ mol^{-1}. The theoretical calculations presented here demonstrate that this assumption is unjustified. Based on the values of the hydration energies determined, the dipole potential values could be recalculated, and they were found to be between 57 and 119 mV more positive in the membrane interior than previously thought.

Although there is no way of electrically directly measuring the value of the dipole potential of a bilayer, another way of estimating its value is to measure the surface potential of a lipid monolayer. This involves spreading the lipid across a clean water surface in a Langmuir trough and measuring the change in electrical potential difference between an electrode located in the aqueous solution below the interface and one in air just above it due to the addition of lipid (Gaines, 1966; Brockman, 1994). The surface potential of a lipid monolayer could be equated with the dipole potential of a bilayer, if one considers a monolayer to be simply one-half of a bilayer. Such measurements are often carried out at a surface pressure of $\sim$30 mN m^{-1}, because this is considered to be the value expected for a biological membrane (Blume, 1979). The dipole potential values determined in this way are generally $\sim$100 to 200 mV higher (Hladky and Haydon, 1973; Beitinger et al., 1989; Smaby and Brockman, 1990) than those previously determined using hydrophobic ions on lipid bilayers. For dioleoylphosphatidylcholine (DOPC), for example, Beitinger et al. (1989) have determined values of 420 and 431 mV at pH 7.4 using two different buffer systems. For egg yolk lecithin (predominant component DOPC) Hladky and Haydon

(1973) determined a value of 441 mV. The conductance measurements of Pickar and Benz (1978) using hydrophobic ion yielded, on the other hand, a value of 224 mV for DOPC (Table 3). This discrepancy between bilayer and monolayer values of the dipole potential has been known for many years, but as yet no generally accepted explanation for it has been found. Smaby and Brockman (1990) have suggested that the discrepancy may be due to an area-independent contribution to the measured monolayer surface potential, because they observed a nonzero intercept in their plots of surface potential against packing density. They proposed that the area-independent contribution might come from a reorganization of the water structure by the lipid head groups. If one corrects the bilayer data for hydration energy differences according to Eq. 7, however, it is found (Table 3) that the discrepancy is significantly reduced. The bilayer value for DOPC becomes, for example, 343 mV, which is much closer to the values of Beitinger et al. (1989) and Hladky and Haydon (1973). If one were to include the additional steric effect of water molecule orientation around the ions in the hydration energy calculation, as discussed by Luzhkov and Warshel (1992), the correction to the bilayer dipole potential data would increase further, and the discrepancy with the monolayer data may vanish completely.

Based on the theoretical calculations presented here, it would seem, therefore, that a correction of hydrophobic ion bilayer conductance data for the different hydration energies of the ions is an essential step for the accurate estimation of the magnitude of the membrane dipole potential. Although the absolute value of the dipole potential can still not be precisely defined, due to the uncertainty in the calculated values of the hydration energies of the hydrophobic ions, the calculations carried out here demonstrate that relatively small differences in the hydration energies of TPB^-, TPP^+, and TPA^+ can easily account for the differences between dipole potential values previously reported from monolayer and bilayer measurements.

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