Origin of membrane dipole potential: Contribution of the phospholipid fatty acid chains

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Abstract

The large intrinsic membrane dipole potential, \( \Phi_d \), is important for protein insertion and functioning as well as for ion transport across natural and model membranes. However, the origin of \( \Phi_d \) is controversial. From experiments carried out with lipid monolayers, a significant dependence on the fatty acid chain length is suggested, whereas in experiments with lipid bilayers, the contribution of additional –CH\(_2\)-groups seems negligibly small compared with that of the phospholipid carbonyl groups and lipid-bound water molecules. To compare the impact of the –CH\(_2\)-groups of dipalmitoylphosphatidylcholine (DPPC) near and far from the glycerol backbone, we have varied the structure of DPPC by incorporation of sulfur atoms in place of methylene groups in different positions of the fatty acid chain. The \( \Phi_d \) of symmetric lipid bilayers containing one heteroatom was obtained from the charge relaxation of oppositely charged hydrophobic ions. We have found that the substitution for a S-atom of a –CH\(_2\)-group decreases \( \Phi_d \). The effect (\( \Delta \Phi_d = -22.6 \text{ mV} \)) is most pronounced for S-atoms near the lipid head group while a S-atom substitution in the C\(_{13}\) or C\(_{14}\)-position of the hydrocarbon chain does not effect the bilayer dipole potential. Most probably \( \Delta \Phi_d \) does not originate from an altered dipole potential of the acyl chain containing an heteroatom but is mediated by the disruption of chain packing, leading to a decreased density of lipid dipoles in the membrane. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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Abbreviations: DPhPC, diphytanoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DHPC, dihexadecylphosphatidylcholine; GMO, glycerolmonooleate; FA, fatty acid; PTFE, polytetrafluoroethylene; IFC, intramembrane field compensation; CR, charge relaxation; MES-4-morpholine ethanesulfonic acid; TPB\(^-\), tetraphenylborate; TPP\(^+\), tetraphenylphosphonium.

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1. Introduction

The electrical profile associated with lipid model or cell membranes is supposed to consist of two main components: the transmembrane potential and the boundary potential. The transmembrane potential is determined by the concentration difference of the ions in the aqueous phase on both sides of the membrane and plays a very important role in the regulation of the function of membrane proteins, especially in the excitable membranes of nerve and heart cells. The boundary potential drop is usually measured at the water–lipid interface and includes at least two subcomponents: a surface potential, well described by Gouy–Chapman theory, and a dipole potential, \( \Phi_d \), existing in the region between the aqueous phase and the hydrocarbon-like interior of the membrane. The latter is always positive and is known to affect ion transport processes across model lipid membranes (Andersen et al., 1976; Flewelling and Hubbell, 1986a). Due to its considerable magnitude (ca. 300 mV in bilayer systems), \( \Phi_d \) has long been supposed to play an important role for protein insertion and functioning (Flewelling and Hubbell, 1986b; Franklin and Cafiso, 1993). Moreover, recent investigations on model systems reveal that dipole potential affects gramicidin channel dissociation (Rokitskaya et al., 1997), modulates the activity of phospholipase A2 (Maggio, 1999), affects the membrane insertion and folding of a model amphiphilic peptide (Cladera and O’Shea, 1998) as well as the extent of the membrane fusion (Cladera et al., 1999).

However, measurements of \( \Phi_d \) in living cells are difficult but have been performed using a ratiometric fluorescent technique (Montana et al., 1989; Gross et al., 1994; Zhang et al., 1998) and cell rotation measurements in the presence of hydrophobic ions (Sukhorukov et al., 2001). Since the results vary between greater than 200 mV using the former technique and approximately 20 mV in the latter, the role of \( \Phi_d \) in cell membrane function is still an open question.

The great potential significance of dipole potential in the regulation of the membrane protein function makes the question about its origin very important. It is now accepted that at least two parameters, the orientation of the phospholipid carbonyl groups and the lipid-bound water molecules, contribute significantly to \( \Phi_d \) (Simon and McIntosh, 1989; Gawrisch et al., 1992; Brockman, 1994). Since the contributions of polarized water and the polar lipid head groups are indistinguishable in most experiments, it was suggested that the contribution from water dipoles is largest to result in the correct (positive) sign of the potential (Gennis, 1989). However, Davies and Rideal (1955) suggested that a third component is required to account for the measured \( \Phi_d \). According to their model, the interface is a three-layer capacitor with a dipole potential, \( \Delta \Phi \):

\[
\Delta \Phi = \frac{12\pi(\mu_1 + \mu_2 + \mu_3)}{A}
\]

where \( \mu_1, \mu_2 \) and \( \mu_3 \) are the apparent partial dipole moments due to water polarization, orientation of the lipid polar head groups and the CH2-bonds of the lipid aliphatic chains, respectively, and \( A \) is the lipid molecular area in Å² per molecule. The various experimental approaches used to evaluate the contribution of the three parameters to \( \Phi_d \) have given conflicting results. Measurements on phospholipid monolayers at the oil–water and air–water interfaces have shown that the contribution of the CH2 groups of the acyl chains of lipids to \( \Phi_d \) is up to 9 mV per CH2-group (Mingins et al., 1992; Evans and Ulman, 1990). However, an analysis by Vogel and Möbius suggests that the value of \( \Delta \Phi \) for zwitterionic lipids and dipalmitylphosphatidylcholine is determined almost exclusively by the terminal methyl group of the aliphatic chain (Vogel and Möbius, 1988; Beitinger et al., 1989). Using an optical method, Clarke (1997) has shown that in the case of unsaturated lipids, the dipole potential depends on the hydrocarbon chain length.

The goal of the present study is to investigate the contribution of the CH2-groups to the dipole potential of lipid bilayers. Variation of the fatty acid structure of synthetic phosphatidylcholines provides a good approach to solve the problem. In the present work we have...
investigated alterations of $\Phi_d$ induced by substitution of a sulfur atom for a methylene group in one of the hydrocarbon chains. By this substitution the geometrical structure of the molecule is slightly altered. The sulfur bond is about 10% longer than a carbon bond (1.68 against 1.53 Å), and the C–S–C angle is somewhat sharper than the C–C–C angle (about 99° against 109°) (Screde et al., 1997). Therefore, the heteroatom-containing lipid is expected to occupy an area that exceeds the area required per DPPC$^1$ molecule. To obtain an upper limit for the incremental area, heteroatom and double bond insertion are compared. At the location of the double bond the acyl chain is tilted and, consequently, tight chain packing is disrupted. Molecular dynamics simulations of bilayers made of dimyristoylphosphatidylcholine (DMPC), 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) or 1-palmitoyl-2-elaidoyl-phosphatidylcholine (PEPC) (Murzyn et al., 2001) show that the area per lipid molecule increases from 60 (for DMPC) to 64 Å (for POPC and PEPC). It can be calculated that the number of lipid dipoles per unit of surface area decreases, thereby, by ca. 7%.

These considerations allow us to distinguish between two hypotheses: (i) methylene group substitution does not change the membrane dipole potential except for the small secondary change ($<20$ mV) introduced by altered lipid packing, which would indicate that phospholipid acyl chains do not contribute to membrane dipole potential; or (ii) methylene group substitution causes changes in the $\Phi_d > 20$ mV, indicating that the acyl chains make a major contribution to membrane dipole potential.

The influence of the S-atom position in the fatty acid chains on $\Phi_d$ are studied here using different DPPC analogs to form planar bilayer membranes and the results were compared with DPPC itself. The $\Phi_d$ was measured with two different methods: charge relaxation in the presence of hydrophobic ions and with the indicator-free capacitance minimization technique.

2. Materials and methods

2.1. Chemicals

For the preparation of buffer solution, 10 mM MES (Boehringer, Mannheim, Germany) and 50 mM KCl (Fluka, Buchs, Switzerland) were used. Tetraphenylborate (TPB$^-$) and tetrphenylphosphonium (TPP$^+$) were obtained from Fluka (Buchs, Switzerland). Dipalmytoylphosphatidylcholine (DPPC) and diphytanoylphosphatidylcholine (DPhPC) were obtained from Avanti Polars Lipids (Alabaster, AL, USA); $n$-decane was purchased from Merck (Germany).

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1 See list of abbreviations.
Fatty acids containing a heteroatom (Fig. 1) were synthesized by replacement of a single CH$_2$-group in different positions by a sulfur atom (Pascal and Ziering, 1986; Lie Ken Jie and Bakare, 1989). The synthetic procedure used to prepare the corresponding DPPC analogues will be presented in a separate publication.

2.2. Membrane formation

Two different techniques of membrane formation were used in this work. Black lipid membranes were formed by the conventional Mueller–Rudin technique in holes ($d = 1$ mm) of a diaphragm of a polytetrafluoroethylene (PTFE) chamber (Mueller et al., 1963). The concentration of membrane-forming lipid was 20 mg lipid/ml $n$-decane. The bilayer surrounding solutions contained typically 10 mM MES and 50 mM KCl and were stirred by magnetic bars. Hydrophobic ions were added from a stock solution in ethanol to both sides of the membrane to reach a final concentration of $10^{-7}$ and $10^{-2}$ M (3 $\times$ $10^{-5}$ M by GMO and DHPC) for TPB$^-$ and TPP$^+$, respectively.

Solvent-free bilayer membranes were formed by the monolayer apposition technique (Montal and Mueller, 1972) in an aperture of a PTFE film separating the two aqueous compartments of a teflon chamber. The aperture had a diameter between 100 and 150 $\mu$m and was made by an electric arc. First the unmodified lipid and the heteroatom-containing lipid dissolved in hexane were placed on the surface of the buffer solutions on the cis and trans sides of the septum, respectively, where they spontaneously formed monolayers. Subsequently, the buffer solution levels in both compartments were raised above the hole using syringes. Thereby, two monolayers merged to a bilayer within the aperture (Fig. 4A). The aperture was pretreated with a hexadecane/hexane solution (volume ratio 1:99).

2.3. Measurements of dipole potential with the charge relaxation method

The dipole potential affects membrane binding and transport of positive and negative ions (Andersen et al., 1978; Andersen and Fuchs, 1975). Consequently, its value can be assessed by CR measurements in the presence of hydrophobic ions (Benz and Gisin, 1978; Benz and Läuger, 1976; Pickar and Benz, 1978). After charging the planar bilayer membrane by short pulses (<1 $\mu$s) to a voltage $V_{m(0)}$ of nearly 10 mV, the time course of membrane voltage $V_{m(t)}$ decay was studied using a setup (Fig. 2) similar to the one described in (Pickar and Benz, 1978). In the presence of negative ions (TPB$^-$), the decay of voltage ratio $V_{m(0)}/V_{m(t)}$ is described by the sum of two exponentials, which are characterized by their relaxation times ($\tau_1$, $\tau_2$) and amplitudes ($a_1$, $a_2$) with $a_1 + a_2 = 1$. In the presence of positive ions (TPP$^+$), only the slow component of the relaxation was observed, i.e. $a_2 = 1$. From an exponential fit to experimental relaxation curves (Fig. 3) obtained in separate experiments for positive and negative ions, $\Phi_d$ was calculated according to Eq. (2) (Pickar and Benz, 1978):

$$\Phi_d = \frac{RT}{2F} \ln \left[ \frac{(k, \beta)^-}{(k, \beta)^+} \right]$$

where $k_j$ and $\beta$ are the rate constants for the translocation of the ion across the inner energy.
barrier and the partition coefficient for ion adsorption at the membrane surface, respectively. The parameters \((k_1\beta_1)\) and \((k_1\beta_2)\) were calculated for positive ions as Eq. (3):

\[
(k_1\beta_1) = \frac{1}{4bc^+\tau_2}
\]  

(3)

and for negative ions as Eq. (4):

\[
(k_1\beta_2) = \frac{N_t}{4c^-\tau_1(1 + bN_t)}
\]  

(4)

where \(c^-\) and \(c^+\) are the final buffer concentrations of TPB\(^-\) and TPP\(^+\), respectively. The total equilibrium concentration of the permeable ion in the membrane, \(N_t\), is calculated as

\[
N_t = \left( \frac{a_1}{b(1 - a_1)} \right)
\]  

(5)

with \(b\):

\[
b = \frac{z^2F^2}{4RTC_m}
\]  

(6)

where \(C_m\) is the specific capacitance of the membrane, \(F\) is the Faraday constant, \(z\) is the valence of the transported ion, \(R\) and \(T\) are the universal gas constant and the temperature, respectively.

2.4. Monitoring of the dipole potential difference between the lipid monolayers using the IFC method

To measure the difference between the boundary potentials Eq. (7) of both membrane leaflets (Fig. 4), we have used the dependence of membrane capacitance on transmembrane potential (Schoch and Sargent, 1976; Schoch et al., 1979). The minimum of capacitance was found by monitoring the second harmonic of the capacitive current (Sokolov and Kuzmin, 1980; Sokolov et al., 1990). The setup was described in our previous work (Pohl et al., 1997, 2000). In brief, the capacitive current of a symmetric bilayer does not contain an overtone (2f) after application of a sine wave with frequency \(f\), which appears only if there is an interleaflet difference of the boundary potential (Fig. 4B). The overtone disappears again if \(\Delta\Phi_b\) is compensated by a d.c.-voltage, \(U\), equal to \(\Delta\Phi_b\) in amplitude but opposite in sign Eq. (7):

\[
U = - (\Delta\Phi_s + \Delta\Phi_d) = - \Delta\Phi_b
\]  

(7)

where \(\Delta\Phi_s\) and \(\Delta\Phi_d\) are the surface and the dipole potential differences between the two leaflets (Fig. 4C), respectively.

The electrical circuit contained three computer-controlled devices: a current to voltage converter (Model 428, Keithley Instruments Inc., Cleveland, OH); a function generator (Model 33120A,
3. Results

3.1. Capacitance measurements of heterolipid membranes

The capacity of solvent-containing heterolipid DPPC membranes was equal to $(0.50 \pm 0.05) \ \mu \text{F cm}^{-2}$. Due to the lack of solvent, a higher capacity of $(0.85 \pm 0.17) \ \mu \text{F cm}^{-2}$ was measured for both symmetric and asymmetric Montal membranes made from the same material. For both types of membranes, the capacity was not distinguishable from that of unlabeled DPPC bilayers.

3.2. $\Phi_d$ measurements on membranes formed from unlabeled DPPC

The absolute dipole potential of unlabeled and sulfur-containing DPPC membranes, $\Phi_d$, was measured by the CR method (Pickar and Benz, 1978). The values recorded for pure lipids (DPPC, DHPC, GMO; Table 1) were found to be in good agreement with values reported earlier (Pickar and Benz, 1978; Gawrisch et al., 1992). $\Phi_d$ of DPhPC was estimated to be $(228 \pm 5) \ \text{mV}$ (Table 1).

3.3. $\Phi_d$ measurements on membranes formed from S-labeled DPPC

A series of DPPC molecules, in which the methylene group at position 3, 4, 7, 13 or 14 of the sn-2 chain was replaced by a sulfur atom, were studied. Fig. 3 shows representative experimental records of CR experiments in the presence of the hydrophobic ions TPB$^-$ ([TPB$^-$] = $10^{-7}$ M, Fig. 3A) and TPP$^+$ ([TPP$^+$] = $10^{-2}$ M, Fig. 3B) obtained with a membrane formed from DPPC,

Hewlett-Packard, Loveland, CO), and a lock-in-amplifier (HMS Elektronik, Leverkusen, Germany) and various custom-made filters.

Table 1

<table>
<thead>
<tr>
<th>Lipid</th>
<th>$\Phi_d$ \ (reference)</th>
</tr>
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<tbody>
<tr>
<td>Dipalmitoylphosphatid</td>
<td>$243 \pm 4 \ \text{mV}$ (DPPC)</td>
</tr>
<tr>
<td></td>
<td>$227 \pm 9 \ \text{mV}$ (Gawrisch et al., 1992)</td>
</tr>
<tr>
<td>Dihexadecylphosphatid</td>
<td>$114 \pm 7 \ \text{mV}$ (DHPC)</td>
</tr>
<tr>
<td></td>
<td>$109 \pm 6 \ \text{mV}$ (Gawrisch et al., 1992)</td>
</tr>
<tr>
<td>Glycerolmonooleolate</td>
<td>$100 \pm 9 \ \text{mV}$ (GMO)</td>
</tr>
<tr>
<td></td>
<td>$108 \ \text{mV}$ (Pickar and Benz, 1978)</td>
</tr>
<tr>
<td>Diphytanoylphosphatid</td>
<td>$228 \pm 5 \ \text{mV}$ (DPhPC)</td>
</tr>
<tr>
<td></td>
<td>$-\text{mV}$</td>
</tr>
</tbody>
</table>

$[\text{TPB}^-] = 10^{-7} \ \text{M}, \ [\text{TPP}^+] = 10^{-2} \ \text{M}$ (DPhPC, DPPC), $[\text{TPP}^+] = 3 \times 10^{-8} \ \text{M}$ (DHPC, GMO). The composition of the buffer solution was 0.05 (DPPC, DPhPC) or 0.1 M (DHPC, GMO) KCl and 20 mM MES, pH 5.5.
where the methylene group at position 7 was replaced by sulfur. Relaxation times and amplitudes for both ions were recorded in subsequent experiments. Based on these parameters, the rate constants Eqs. (3) and (4) and $\phi_d$ were calculated.

A concentration interval was chosen such that the product of the parameters $k$ and $\beta$ did not depend on the concentration of the hydrophobic ions. The substitution of the methylene groups by sulfur atoms altered the transport parameters of both ions. Measurements on lipid membranes show that $\phi_d$ decreased significantly (Fig. 5, black squares). The magnitude of this effect depended on the position of the S-atom in the fatty acid chain. It was most pronounced ($\Delta \phi_d = -22.6$ mV) for S-atoms closest to the glycerol backbone (substitution of the 3rd or 4th methylene group) and decreased toward the methyl terminus of the hydrocarbon chain.

To confirm the results obtained with the CR technique, measurements with the IFC method were undertaken. Solvent-free bilayer membranes were formed by the monolayer apposition technique (Montal and Mueller, 1972). The cis-monolayer was made from DPhPC, the trans-monolayer from the S-containing DPPC. The interleaflet boundary potential difference was measured. The IFC results were in good agreement with the CR recordings (Fig. 5, grey squares).

### 4. Discussion

The substitution of S atoms for methylene groups in the fatty acid chain of a DPPC bilayer decreases membrane dipole potential as revealed by charge relaxation of planar bilayers doped with hydrophobic ions. Since the energy barrier for the transmembrane movement of positively and negatively charged hydrophobic ions is affected differently, this effect cannot be explained by simple defects in the lipid packing introduced by the heteroatom. Rather it is the total dipole potential generated by the lipid molecules and the lipid-bound water that is affected. This conclusion is confirmed by direct measurements of the interleaflet dipole potential difference of a planar membrane in which only one of the leaflets is made of the heteroatom-containing lipid.

The indicator-free IFC technique used here allows the resolution of differences in the dipole potential that are smaller than 1 mV. Consequently, it can be concluded that the changes of the dipole potential measured here are significant. However, the drop of $\phi_d$ does not exceed 20 mV which is small compared with the effect induced by the alignment of small dipoles, as for example phloretin (Franklin and Cafiso, 1993; Forman et al., 1985; Cseh and Benz, 1999; Pohl et al., 1997), verapamil (Pohl et al., 1998) and ketocholestanol (Franklin and Cafiso, 1993) into the bilayer. The observed $\phi_d$ alteration can be explained by changed chain packing that is accompanied by an increase in the area per lipid molecule, e.g. a decrease of the number of dipoles per unit area. If acyl chains contribute significantly to membrane dipole potential, larger deviations between the dipole potentials of membranes made of DPPC and of S-atom substituted DPPC should be expected.

With increasing distance from the head group, the potential of the sulfur atom to increase the area per lipid molecule is reduced. Since the lipid order parameter decreases in the same direction (Seelig and Browning, 1978; Huster et al., 1998; Holte et al., 1995), this result was expected. A distant modification in acyl chain structure is not anticipated to disturb chain packing because distant methylene groups are allowed to move more
freely. That applies to the terminal methyl group, too; although possessing a dipole moment of 0.35 D (Vogel and Mobius, 1988), it can not contribute to the time-averaged membrane dipole potential to the same extent as an aligned dipole close to the interface because it has a more random orientation. Following this line of reasoning, any dependency of the membrane dipole potential magnitude should become smaller with increasing FA chain length.

The dependencies of both $\Delta \Phi_d$ and the order parameter on the respective position of the S-atoms have qualitatively different shapes. The rather linear relationship between $\Delta \Phi_d$ and sulfur atom position shown in Fig. 5 also suggests that packing effects interfere. Support for this idea comes from the observation that the replacement of a single methylene group in the lipid fatty acid chain by sulfur atoms lowers the phase transition temperature significantly (Mannock et al., 1999). These results show that the disruption of the chain packing in lipids containing heteroatom-substituted acyl chains is comparable to that observed by the introduction of a single double bond into a lipid containing $n$-saturated acyl chains. Increasing unsaturation, in turn, is known to decrease the value of the $\Phi_d$ (Clarke, 1997), probably because of the effects of chain packing on the spacing between the polar headgroups. Similarly, heteroatoms are expected to increase the area per lipid molecule, i.e. to decrease the number of dipoles per unit of membrane surface and thereby to diminish membrane dipole potential (compare Eq. (1)).

In summary, we conclude that an important contribution of the acyl chains to membrane dipole potential arises from its function as a determinant of lipid packing density. Any contribution behind the steric one, e.g. an input of its C–H bonds to the dipole potential, cannot be deduced from substitution experiments of the type reported here.

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