

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

Uwe Peterson^a, David A. Mannock^b, Ruthven N.A.H. Lewis^b, Peter Pohl^{a,c},
Ronald N. McElhaney^b, Elena E. Pohl^{a,d,*}

^a *Institut für Medizinische Physik und Biophysik, Martin-Luther-Universität, 06097 Halle, Germany*

^b *Department of Biochemistry, University of Alberta, Edmonton, Alta., Canada T6G 2H7*

^c *Forschungsinstitut für Molekulare Pharmakologie, 13125 Berlin, Germany*

^d *Institut für Physiologie, Otto-von-Guericke Universität, Leipziger Str. 44, D-39120 Magdeburg, Germany*

Received 28 November 2001; received in revised form 15 February 2002; accepted 20 February 2002

Abstract

The large intrinsic membrane dipole potential, Φ_d , is important for protein insertion and functioning as well as for ion transport across natural and model membranes. However, the origin of Φ_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 $-\text{CH}_2$ -groups seems negligibly small compared with that of the phospholipid carbonyl groups and lipid-bound water molecules. To compare the impact of the $-\text{CH}_2$ -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 Φ_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 $-\text{CH}_2$ -group decreases Φ_d . The effect ($\Delta\Phi_d = -22.6$ 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.

Keywords: Charge relaxation; Capacitance minimization; Tetrphenylborate; Tetrphenylphosphonium

Abbreviations: DPhPC, diphtanoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DHPC, dihexadecylphosphatidylcholine; GMO, glycerolmonooleate; FA, fatty acid; PTFE, polytetrafluoroethylene; IFC, intramembrane field compensation; CR, charge relaxation; MES-4-morpholine ethanesulfonic acid; TPB^- , tetrphenylborate; TPP^+ , tetrphenylphosphonium.

* Corresponding author. Tel.: +49-391-6713695; fax: +49-391-6715819

E-mail address: elena.pohl@medizin.uni-magdeburg.de (E.E. Pohl).

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, Φ_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), Φ_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 Φ_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 Φ_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 Φ_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 Φ_d . According to their model, the interface is a three-layer capacitor with a dipole potential, $\Delta\Phi$:

$$\Delta\Phi = \frac{12\pi(\mu_1 + \mu_2 + \mu_3)}{A} \quad (1)$$

where μ_1 , μ_2 and μ_3 are the apparent partial dipole moments due to water polarization, orientation of the lipid polar head groups and the CH₃-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 Φ_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 Φ_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 dipalmitoylphosphatidylcholine 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 Φ_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 DPPC¹ 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 Φ_d are studied here using different DPPC analogs to form planar bilayer membranes and the results were compared with DPPC itself. The Φ_d was measured with two different methods: charge relaxation in the presence of hydrophobic ions and with the indicator-free capacitance minimization technique.

¹ See list of abbreviations.

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 tetraphenylphosphonium (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).

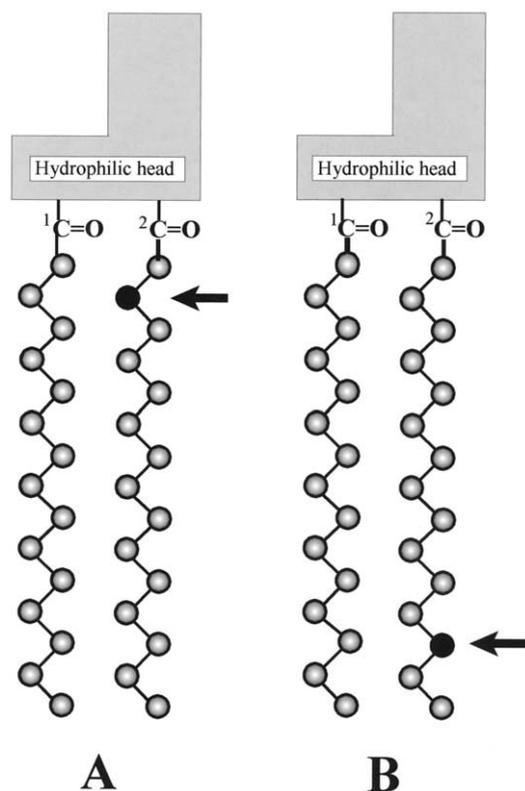


Fig. 1. The structure of sulfur containing DPPC analogues. The arrows indicate the position of the sulfur atom. The numbers 1 and 2 denote fatty acyl chains at positions sn-1 and sn-2 of the glycerol moiety. (A) 1-palmitoyl, 2-(2-S-13-thia-palmitoyl)-phosphatidylcholine (the S substitution is at carbon atom 3). (B) 1-palmitoyl, 2-(13-S-2-thia-palmitoyl)-phosphatidylcholine (the S substitution is at carbon atom 14).

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 (3×10^{-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 μm 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 (An-

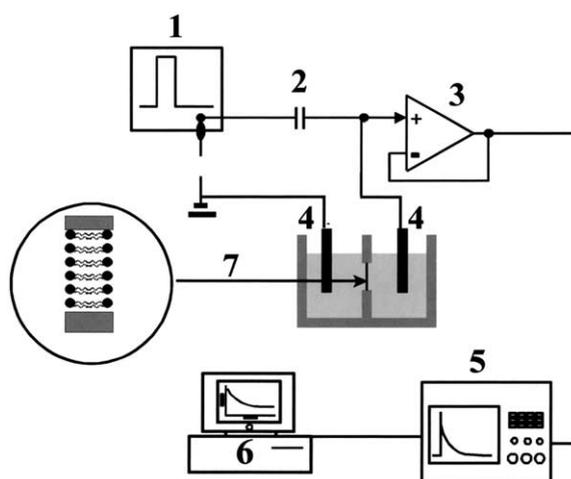


Fig. 2. Experimental arrangement for dipole potential measurements with the CR method. 1, Pulse generator; 2, Coupling capacity; 3, Amplifier; 4, Electrodes; 5, Oscilloscope; 6, PC; 7, Symmetric bilayer membrane made from P(x-S-y)PC monolayers.

dersen 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 Lauger, 1976; Pickar and Benz, 1978). After charging the planar bilayer membrane by short pulses ($< 1 \mu\text{s}$) 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 (τ_1 , τ_2) and amplitudes (a_1 , a_2) with $\tau_1 \ll \tau_2$ and $a_1 + a_2 = 1$. 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, Φ_d was calculated according to Eq. (2) (Pickar and Benz, 1978):

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

where k_i and β 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 $(k_i\beta)^+$ and $(k_i\beta)^-$ were calculated for positive ions as Eq. (3):

$$(k_i\beta)^+ = \frac{1}{4bc^+\tau_2} \quad (3)$$

and for negative ions as Eq. (4):

$$(k_i\beta)^- = \frac{N_t}{4c^-\tau_1(1 + bN_t)} \quad (4)$$

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

$$N_t = \left(\frac{a_1}{b(1 - a_1)} \right) \quad (5)$$

with b :

$$b = \frac{z^2F^2}{4RTC_m} \quad (6)$$

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):

$$U = -(\Delta\Phi_s + \Delta\Phi_d) = -\Delta\Phi_b \quad (7)$$

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,

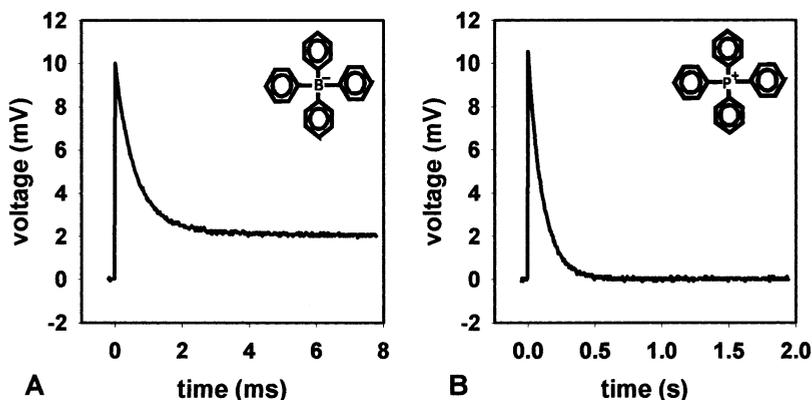


Fig. 3. Representative traces of charge relaxation. Planar membranes were made from 1-palmitoyl, 2-(6-S-9-thiapalmitoyl)-phosphatidylcholine. (A) In the presence of TPB^- ($c = 10^{-7}$ M) relaxation time and amplitude were, respectively, $\tau_1 = 0.56$ ms, $a_1 = 0.82$. (B) In the presence of TPP^+ ($c = 10^{-2}$ M) the relaxation time was equal to $\tau_2 = 117$ ms. The buffer solution contained 50 mM KCl, 10 mM MES (pH 5.5, temperature = 50 °C).

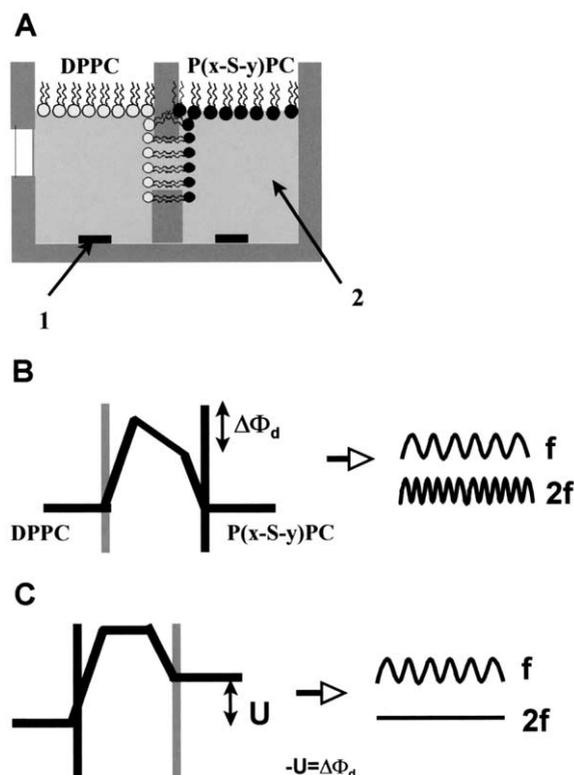


Fig. 4. Principle of the intramembrane field compensation method. (A) A bilayer consisting of two different monolayers (*cis*-DPPC, *trans*-P(x-S-y)PC) forms spontaneously after raising of buffer solution levels above the aperture. (B) Due to an interleaflet difference of the boundary potential, an overtone ($2f$) of the capacitive current appears after application of a sine wave with frequency f . (C) The overtone disappears again if $\Delta\Phi_d$ is compensated by a d.c.-voltage equal to $\Delta\Phi_d$ in amplitude but opposite in sign. 1, stirrer bar; 2, buffer solution.

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

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. Φ_d measurements on membranes formed from unlabeled DPPC

The absolute dipole potential of unlabeled and sulfur-containing DPPC membranes, Φ_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). Φ_d of DPhPC was estimated to be $(228 \pm 5) \text{ mV}$ (Table 1).

3.3. Φ_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⁻ ($[\text{TPB}^-] = 10^{-7} \text{ M}$, Fig. 3A) and TPP⁺ ($[\text{TPP}^+] = 10^{-2} \text{ M}$, Fig. 3B) obtained with a membrane formed from DPPC,

Table 1

The dipole potential of different lipids measured with the CR method

Lipid	Φ_d	Φ_d (reference)
Dipalmitoylphosphatidylcholine (DPPC)	$243 \pm 4 \text{ mV}$	$227 \pm 9 \text{ mV}$ (Gawrisch et al., 1992)
Dihexadecylphosphatidylcholine (DHPC)	$114 \pm 7 \text{ mV}$	$109 \pm 6 \text{ mV}$ (Gawrisch et al., 1992)
Glycerolmonooleate (GMO)	$100 \pm 9 \text{ mV}$	108 mV (Pickar and Benz, 1978)
Diphytanoylphosphatidylcholine (DPhPC)	$228 \pm 5 \text{ mV}$	–

$[\text{TPB}^-] = 10^{-7} \text{ M}$, $[\text{TPP}^-] = 10^{-2} \text{ M}$ (DPhPC, DPPC), $[\text{TPP}^-] = 3 \times 10^{-5} \text{ M}$ (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.

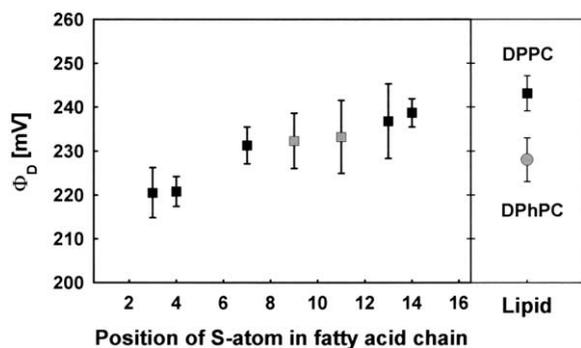


Fig. 5. Dependence of Φ_d on the position of the S-atom in the fatty acid chain of DPPC. Buffer solution contained 50 mM KCl, 10 mM MES (pH 5.5, temperature = 50 °C).

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 Φ_d were calculated.

A concentration interval was chosen such that the product of the parameters k and β 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 Φ_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 Φ_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 Φ_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 Φ_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.

Acknowledgements

Supported by Kultusministerium Sachsen-Anhalt, Germany (to E.E. Pohl), FKZ 2884A/0028G and Deutsche Forschungsgemeinschaft Po 533/7-1 (to P. Pohl), the Canadian Institute of Health

Research and the Alberta Heritage Foundation for Medical Research (to R.N. McElhoney).

References

- Andersen, O.S., Fuchs, M., 1975. Potential energy barriers to ion transport within lipid bilayers. *Biophys. J.* 15, 795–830.
- Andersen, O.S., Finkelstein, A., Katz, I., Cass, A., 1976. Effect of phloretin on the permeability of thin lipid membranes. *J. Gen. Physiol.* 67, 749–771.
- Andersen, O.S., Feldberg, S., Nakadomary, H., Levy, S., McLaughlin, S., 1978. Electrostatic interactions among hydrophobic ions in lipid bilayer membranes. *Biophys. J.* 21, 35–70.
- Beitinger, H., Vogel, V., Mobius, D., Rahmann, H., 1989. Surface potentials and electric dipole moments of ganglioside and phospholipid bilayers: contribution of the polar headgroup at the water/lipid interface. *Biochim. Biophys. Acta* 984, 293–300.
- Benz, R., Läuger, P., 1976. Kinetic analysis of carrier-mediated ion transport by charge-pulse technique. *J. Membr. Biol.* 27, 171–191.
- Benz, R., Gisin, B.F., 1978. Influence of membrane structure on ion transport through lipid bilayer membranes. *J. Membr. Biol.* 40, 293–314.
- Brockman, H., 1994. Dipole potential of lipid membranes. *Chem. Phys. Lipids* 73, 57–79.
- Cladera, J., O'Shea, P., 1998. Intramembrane molecular dipoles affect the membrane insertion and folding of a model amphiphilic peptide. *Biophys. J.* 74, 2434–2442.
- Cladera, J., Martin, I., Ruyschaert, J.M., O'Shea, P., 1999. Characterization of the sequence of interactions of the fusion domain of the simian immunodeficiency virus with membranes. Role of the membrane dipole potential. *J. Biol. Chem.* 274, 29951–29959.
- Clarke, R.J., 1997. Effect of lipid structure on the dipole potential of phosphatidylcholine bilayers. *Biochim. Biophys. Acta* 1327, 269–278.
- Cseh, R., Benz, R., 1999. Interaction of phloretin with lipid monolayers: relationship between structural changes and dipole potential change. *Biophys. J.* 77, 1477–1488.
- Davies, J.T., Rideal, E., 1955. Interfacial potentials. *Can. J. Chem.* 33, 947–960.
- Evans, S.D., Ulman, A., 1990. Surface potential studies of alkyl-thiol monolayers adsorbed on gold. *Chem. Phys. Lett.* 170, 462–466.
- Flewelling, R.F., Hubbell, W.L., 1986a. Hydrophobic ion interactions with membranes. Thermodynamic analysis of tetraphenylphosphonium binding to vesicles. *Biophys. J.* 49, 531–540.
- Flewelling, R.F., Hubbell, W.L., 1986b. The membrane dipole potential in a total membrane potential model. Applications to hydrophobic ion interactions with membranes. *Biophys. J.* 49, 541–552.

- Forman, S.A., Verkman, A.S., Dix, J.A., Solomon, A.K., 1985. *n*-Alkanols and halothane inhibit red cell anion transport and increase band 3 conformational change rate. *Biochemistry* 24, 4859–4866.
- Franklin, J.C., Cafiso, D.S., 1993. Internal electrostatic potentials in bilayers: measuring and controlling dipole potentials in lipid vesicles. *Biophys. J.* 65, 289–299.
- Gawrisch, K., Ruston, D., Zimmerberg, J., Paresgian, V.A., Rand, R.P., Fuller, N., 1992. Membrane dipole potentials, hydration forces, and the ordering of water at membrane surfaces. *Biophys. J.* 61 (5), 1213–1223.
- Gennis, R.B., 1989. *Biomembranes. Molecular Structure and Function*. Springer, New York, Berlin.
- Gross, E., Bedlack, R.S.J., Loew, L.M., 1994. Dual-wavelength ratiometric fluorescence measurement of the membrane dipole potential. *Biophys. J.* 67, 208–216.
- Holte, L.L., Peter, S.A., Sinnwell, T.M., Gawrisch, K., 1995. ²H nuclear magnetic resonance order parameter profiles suggest a change of molecular shape for phosphatidylcholines containing a polyunsaturated acyl chain. *Biophys. J.* 68, 2396–2403.
- Huster, D., Arnold, K., Gawrisch, K., 1998. Influence of docosahexaenoic acid and cholesterol on lateral lipid organization in phospholipid mixtures. *Biochemistry* 37, 17299–17308.
- Lie Ken Jie, M.S.F., Bakare, O., 1989. H-1 and C-13 NMR—studies on the positional isomers of methyl thialauzate and methyl thiastearate. *J. Chem. Soc. Perkin Trans. II*, 2121–2125.
- Maggio, B., 1999. Modulation of phospholipase A2 by electrostatic fields and dipole potential of glycosphingolipids in monolayers. *J. Lipid Res.* 40, 930–939.
- Mannock, D.A., Lewis, R.N.A.H., McElhaney, R.N., 1999. Thermotropic characterisation of lipid containing alkyl thia- and alkyl seleno fatty acids: model lipids for X-ray diffraction and imaging studies. *Biophys. J.* 76, A431.
- Mingins, J., Stigter, D., Dill, K.A., 1992. Phospholipid interactions in model membrane systems. I. Experiments on monolayers. *Biophys. J.* 61, 1603–1615.
- Montal, M., Mueller, P., 1972. Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. *Proc. Natl. Acad. Sci. USA* 69, 3561–3566.
- Montana, V., Farkas, D.L., Loew, L.M., 1989. Dual-wavelength ratiometric fluorescence measurements of membrane potential. *Biochemistry* 28, 4536–4539.
- Mueller, P., Rudin, D.O., Tien, H.T., Wescott, W.C., 1963. Methods for the formation of single bimolecular lipid membranes in aqueous solution. *J. Phys. Chem.* 67, 534–535.
- Murzyn, K., Rog, T., Jezierski, G., Takaoka, Y., Pasenkiewicz-Gierula, M., 2001. Effects of phospholipid unsaturation on the membrane/water interface: amolecular simulation study. *Biophys. J.* 81, 170–183.
- Pascal, R.A. Jr, Ziering, D.L., 1986. Synthesis of heteroatom-substituted analogues of stearic acid. *J. Lipid Res.* 27, 221–224.
- Pickar, A.D., Benz, R., 1978. Transport of oppositely charged lipophilic probe ions in lipid bilayer membranes having various structures. *J. Membr. Biol.* 44, 353–376.
- Pohl, P., Rokitskaya, T.I., Pohl, E.E., Saparov, S.M., 1997. Permeation of phloretin across bilayer lipid membranes monitored by dipole potential and microelectrode measurements. *Biochim. Biophys. Acta* 1323, 163–172.
- Pohl, E.E., Krylov, A.V., Block, M., Pohl, P., 1998. Changes of the membrane potential profile induced by verapamil and propranolol. *Biochim. Biophys. Acta* 1373, 170–178.
- Pohl, E.E., Peterson, U., Sun, J., Pohl, P., 2000. Changes of intrinsic membrane potentials induced by flip-flop of long-chain fatty acids. *Biochemistry* 39, 1834–1839.
- Rokitskaya, T.L., Antonenko, Y.N., Kotova, E.A., 1997. Effect of the dipole potential of a bilayer lipid membrane on gramicidin channel dissociation kinetics. *Biophys. J.* 73, 850–854.
- Schoch, P., Sargent, D.F., 1976. Surface potentials of asymmetric charged lipid bilayers. *Experientia* 32, 811.
- Schoch, P., Sargent, D.F., Schwyzer, R., 1979. Capacitance and conductance as tools for the measurement of asymmetric surface potentials and energy barriers of lipid bilayer membranes. *J. Membr. Biol.* 46, 71–89.
- Screde, S., Sorensen, H.N., Larsen, L.N., Steineger, H.H., Hovik, K., Spydevold, O.S., Horn, R., Bremer, J., 1997. Thia fatty acids, metabolism and metabolic effects. *Biochem. Biophys. Acta* 1344, 115–131.
- Seelig, J., Browning, J.L., 1978. General features of phospholipid conformation in membranes. *FEBS Lett.* 92, 41–44.
- Simon, S.A., McIntosh, T.J., 1989. Magnitude of the solvation pressure depends on dipole potential. *Proc. Natl. Acad. Sci. USA* 86, 9263–9267.
- Sokolov, V.S., Kuzmin, V.G., 1980. Measurement of differences in the surface potentials of bilayer membranes according to the second harmonic of a capacitance current. *Biofizika* 25, 170–172 In Russian.
- Sokolov, V.S., Cherny, V.V., Simonova, M.V., Markin, V.S., 1990. Electrical potential distribution over the bilayer lipid membrane due to amphiphilic ion adsorption. *Bioelectrochem. Bioenerg.* 23, 27–44.
- Sukhorukov, V.L., Kürschner, M., Dilsky, S., Lisec, T., Wagner, B., Schenk, W.A., Benz, R., Zimmermann, U., 2001. Phloretin-induced changes of lipophilic ion transport across the plasma membrane of mammalian cells. *Biophys. J.* 81 (2) 1006–1013.
- Vogel, V., Mobius, D., 1988. Local surface potentials and electric dipole moments of lipid monolayers: contributions of the water/lipid and the lipid/air interfaces. *J. Coll. Interf. Sci.* 126, 408–420.
- Zhang, J., Davidson, R.M., Wei, M.D., Loew, L.M., 1998. Membrane electric properties by combined patch clamp and fluorescence ratio imaging in single neurons. *Biophys. J.* 74, 48–53.