Permeability and Electrical Properties of Planar Lipid Membranes from Thylakoid Lipids

Bruno Fuks and Fabrice Homblé
Laboratoire de Physiologie Végétale, Faculté des Sciences, Université Libre de Bruxelles, Brussels, Belgium

ABSTRACT Electrical measurements were carried out on planar lipid membranes from thylakoid lipids. The specific capacitance of membranes formed from decane-containing monogalactosyldiacylglycerol (MGDG), which accounts for 57% of the total lipid content of thylakoids, showed that it adopted a bilayer structure. Solvent-free bilayers of MGDG were not formed, with very rare exceptions, indicating that decane is required to stabilize the planar conformation. However, this cone-shaped lipid produces bilayer structures in combination with other cylindrical thylakoid lipids even in the absence of organic solvent. We compared the properties of solvent-free and decane-containing bilayers from MGDG, soybean lecithin, and the quaternary mixture of lipids similar to that found in vivo. The conductance of decane-MGDG was 26 times higher than that of decane-lecithin. The flux through the decane-lecithin bilayer was found to be slightly dependent on pH, whereas the decane-MGDG membrane was not. The specific conductance of bilayers formed from the quaternary mixture of lipids was 5 to 10 times larger than lecithin (with alkane or not). Further experiments with bilayers made in the presence of a KCl gradient showed that decane-MGDG, decane-MGDG/DGDG/SQDG/PG, and solvent-free MGDG/DGDG/SQDG/PG were cation-selective. The permeability coefficient for potassium ranged from 4.9 to 8.3 $\times$ 10^{-11} cm s^{-1}. The permeability coefficient for protons in galactolipids, however, was determined to be about six orders of magnitude higher than the value for potassium ions. The HCl permeation mechanism through the lipid membranes was determined from diffusion potentials measured in HCl gradients. Our results suggest that HCl was not transported as neutral molecules. The data is discussed with regard to the function of galactolipids in the ion transport through thylakoid membranes.

INTRODUCTION

The lipid composition of thylakoid membrane is distinct from that of other biological membranes. The dominating species is the uncharged glycolipid monogalactosyldiacylglycerol (MGDG) with a 57% by weight. The second most important lipid is digalactosyldiacylglycerol (DGDG), which represents 27% of the total lipid (Dorne et al., 1990). The remaining classes consist of the anionic lipids sulphoquinovosyldiacylglycerol (SQDG) (7%), phosphatidylglycerol (7%), and phosphatidylinositol (2%). A property of MGDG is that it adopts a nonbilayer configuration when dispersed in an aqueous phase. Under certain conditions of temperature and concentration, the MGDG adopts the hexagonal H_{II} phase encapsulating cylinders of water. In contrast, DGDG, SQDG, and PG prefer the conventional lipid bilayer structures when hydrated (Shipley et al., 1973; Gounaris et al., 1983; Sprague and Stahelin, 1984; Webb and Green, 1991). When MGDG is mixed into other thylakoid lipids by conventional dispersal method, freeze-fracture electron micrographs of liposomes show that it forms inverted micelles inserted between the leaflets of lipid bilayers (Gounaris et al., 1983; Sprague and Stahelin, 1984). In contrast, Webb and Green (1989) obtained extruded liposomes composed of MGDG/DGDG/SQDG/PG that lack both the H_{II} phase and inverted lipid particles. Moreover, inverted micelles or tubes have not been observed in the native membrane unless the membrane is stressed (Webb and Green, 1991). The MGDG painted across a teflon hole can form a black lipid membrane (BLM) without the addition of other lipids (Graziani and Livne, 1972), but the role of alkanes was not investigated. These results indicate that the possible existence of nonbilayer phases in purified lipid mixtures or in native membrane is not clear. Therefore, further research is required to answer this question.

In chloroplasts, light-driven H^+ flux is electrically compensated by inward movements of Cl^- and efflux of both K^+ and Mg^{2+} (Hind et al., 1974; Vredenberg, 1976; Junge, 1977). Although initially an electrical membrane potential difference precedes the formation of a pH gradient, the induced secondary transport of ions decreases this electrical potential difference (around +30 mV) so that a pH gradient (pH = 8 outside, pH = 5 inside) is built up. The molecular basis of this ionic flux is unresolved, especially with regard to the permeability of thylakoid lipids. By analogy with what is known for membranes formed from phospholipid, we expect that the lipid permeability of thylakoid would be low to maintain an electrochemical potential gradient. Indeed, the permeability of phospholipid bilayers is in the range between 10^{-10} and 10^{-12} cm s^{-1} for K^+, Na^+, and Cl^- (Toyoshima and Thompson, 1975; Nichols and Deamer, 1980; Gutknecht and Walter, 1981a). Because knowledge of the proton permeability coefficient is important to verify Mitchell's chemiosmotic theory, several studies have attempted to answer this question. Surprisingly high values of permeability coefficients ranging from 10^{-5} to 10^{-3} cm s^{-1} were measured in artificial bilayers made from various phospholipids at neutral

Received for publication 8 June 1993 and in final form 11 February 1994.
Address reprint requests to Bruno Fuks, Laboratoire de Physiologie Végétale, Faculté des Sciences C.P. 206/2, Université Libre de Bruxelles, Campus Plaine, B-1050 Brussels, Belgium. Tel: 32-2-6505383; Fax: 32-2-6505113.

0006-3495/94/05/1404/11 $2.00

pH (Nichols and Deamer, 1980; Deamer and Nichols, 1983; Gutknecht, 1984; Deamer, 1987). Little work has been done in this field on thylakoid lipids: their H^+ permeability is unknown, and results for K^+ and Cl^- are contradictory. Foley et al. (1988) reported that liposomes made from a mixture of DGDG and MGDG have the same permeabilities to KCl as those of PC liposomes. However, the salt concentrations used in their work are known to induce aggregation of liposomes. Measurements made by Webb and Green (1989), at lower salt concentrations, showed that large unilamellar liposomes formed from DGDG were 100-fold more permeable to Rb^+ and at least 50-fold less permeable to Cl^- than PC liposomes. Their results also demonstrated that a lipid composition similar to the one found in vivo proved to be two orders of magnitude more permeable to Rb^+ than PC liposomes; i.e., $P_{Rb} = 2 \times 10^{-9}$ cm s^{-1}. Webb and Green (1989, 1991) have concluded that galactolipids play a major role in the thylakoid membrane because they are a significant diffusive pathway for ions.

In this paper we describe some electrical properties of planar lipid bilayers formed from MGDG and from thylakoid lipid mixtures with or free of n-decane. The absolute permeability coefficient for potassium, chloride, and proton is calculated from experimental data. The possible influence of decane on the measurements was also investigated. The results presented in this work show that decane-MGDG and thylakoid lipid mixtures are more conductive than phospholipids. Their contribution to passive ion flux through the thylakoid membrane is discussed.

MATERIALS AND METHODS

Materials

The bilayers were formed from galactolipids (MGDG, DGDG, and SQDG) and PG extracted from higher plant chloroplasts (Lipid Products, Redhill, UK) and from soybean lecithin type II-S (Avanti Polar Lipids, Inc., Pelham, AL). Solutions of galactolipids in chloroform/methanol (2:1, v/v) were evaporated to dryness under N_2. Lipids (10 mg) were then dissolved in 1 ml of n-decane, which was more than 99% pure (Merck, Darmstadt, Germany). All other chemicals employed were analytical reagent grade. The samples of soybean lecithin were prepared by dissolving 15 mg of phospholipids in 1 ml of n-decane. Solutions were made using triple-distilled water and were filtered through 0.2 μm Acrodisc filters (Gelman Sciences, Ann Arbor, MI) before use.

Planar lipid membranes

Black lipid membranes in aqueous solutions were formed by the technique of Mueller et al. (1962). In brief: a teflon beaker set or a styrene-copolymer cup was immersed in the appropriate salt solution inside a glass cup. The bilayers were prepared by painting a membrane-forming solution over the hole of the septum (teflon: 1 mm^2 in area; plastic: 0.03 mm^2). Before the vessel was immersed in the aqueous solution, a small part around the hole was smeared with membrane solution to facilitate the formation of the bilayers.

Solvent-free bilayers were made by bringing together two monolayers of lipids (1% in hexane) over a hole (200 μm in diameter) in a thin sheet (25 μm thick) of Teflon treated with squalene (Montal and Mueller, 1972). The solvent was evaporated at least 5 min before the folding of the monolayers.

Electrical recordings

The two aqueous phases separated by the lipid film are defined as *cis* and *trans* compartments. The *cis* compartment is the one to which a voltage generator is connected to the bilayers through an Ag/AgCl electrode. The *trans* compartment is connected to the ground through a second Ag/AgCl electrode. Both electrodes are connected to the chambers through 3 M KCl agar bridges. The formation of the bilayer membrane was checked by continuously following the membrane capacitance. This was measured by applying rectangular voltage pulses (50 or 100 mV) of 10 ms duration through silver-silver chloride electrodes and recording directly the capacitive current on an oscilloscope. Current was measured with a current-voltage converter (Bio-Logic RK-300, Claix, France). Current through and potential across the lipid film were known, and the specific conductance of the membrane was defined by Ohm's law.

To measure diffusion potentials produced by KCl or HCl gradients, BLM was formed with both aqueous phases at identical concentration, and the electrolyte concentration in the *cis* compartment was then altered by the addition of more concentrated salt with constant stirring. To measure H^+/OH^- conductance and permeability, avoiding background effects produced by other ions, ionically balanced buffer mixtures were used as described in detail by Gutknecht (1984). Planar bilayers were formed in symmetrical concentrations of buffers on both sides (30 mM HEPES plus 30 mM TRIS or 30 mM MES plus 30 mM BIS-TRIS). A pH gradient was produced by adding 20 mM HEPES (or MES) to the *cis* side and 20 mM TRIS (or BIS-TRIS) to the *trans* side. The transference number of H^+/OH^- can be calculated from the diffusion potential measured in the pH gradient because there are no gradients of buffer cation and anion. The pH in the two chambers was monitored before and after each experiment and was found to be stable. Experimentally, it is not possible to distinguish between H^+ and OH^- permeability coefficients, so this is indicated as (H^+/OH^-). We will refer to this as proton permeability P_H.

Data are presented in the form of mean ± SEM (n = number of replicates). Statistical significance refers to a Student's test ($p < 0.05$). The experiments were carried out at room temperature.

RESULTS

Specific capacitance and thickness of bilayers

By dissolving MGDG or a mixture of MGDG/DGDG/SQDG/PG (2:1:0.5:0.5, w/w) in n-decane, we were able to form stable planar bilayers. The mechanical stability and the lifetimes of the bilayers were as good as soybean lecithin bilayers. The best method for assessing the state of bilayers is to measure its specific electrical capacitance. The measurements showed that the various lipid films were a bimolecular structure. Indeed, in the case of Mueller-Rudin BLM, all values were in the range of 0.33 to 0.41 μF cm^{-2} and were constant for each membrane over its lifetime (Table 1). Table 1 gives also the hydrocarbon thickness, d, of the membrane, which is calculated from the relation $C = \epsilon_0\epsilon/d$, where $\epsilon_0 = 8.85 \times 10^{-12}$ F m^{-1} is the permitivity of free space and $\epsilon = 2.1$ is the average dielectric constant of a long-chain hydrocarbon (Benz et al., 1975). From the specific membrane capacitance, we have calculated that a Mueller-Rudin BLM formed from galactolipids should have an overall thickness of about 5 nm. The values of capacitance were unaffected by the nature and concentration of surrounding salt solution (Table 1). The thickness of planar bilayers formed from thylakoid lipids and phospholipids were similar. The solvent-free bilayers from MGDG/DGDG/SQDG/PG made by bringing together two monolayers showed a specific capacitance around 0.85 μF cm^{-2}. We were able to produce that

TABLE 1 Specific capacitance, C_m, and hydrocarbon thickness, d, of planar lipid bilayers from MGDG, MGDG/DGDG/SQDG/PG, and soybean lecithin

Salt concentration	C_m	d	Number of membranes
mM	$\mu F\ cm^{-2}$	nm	
	Decane-MGDG bilayers		
10 KCl	0.36 ± 0.02	5.2	10
150 KCl	0.33 ± 0.01	5.6	5
500 KCl	0.35 ± 0.02	5.3	6
500 NaCl	0.34 ± 0.02	5.5	4
500 LiCl	0.39 ± 0.01	4.8	5
	Solvent-free MGDG bilayers		
500 KCl	0.73 ± 0.06	2.6	3*
	Decane-MGDG/DGDG/SQDG/PG bilayers		
10 KCl	0.39 ± 0.03	4.8	6
500 KCl	0.41 ± 0.01	4.5	3
	Solvent-free MGDG/DGDG/SQDG/PG bilayers		
1 HCl	0.89 ± 0.01	2.1	11
1 KCl	0.87 ± 0.03	2.1	3
10 KCl	0.85 ± 0.10	2.2	5
500 KCl	0.84 ± 0.01	2.2	3
	Decane-soybean lecithin bilayers		
500 KCl	0.34 ± 0.01	5.5	25
	Solvent-free soybean lecithin bilayers		
10 KCl	0.83 ± 0.06	2.2	6
500 KCl	0.80 ± 0.03	2.3	19

* The formation of solvent-free bilayers from MGDG was successful in less than 5% of trials.

type of bilayer by using MGDG alone in about 5% of the trials. The solvent-free lecithin bilayers exhibited also a higher specific capacitance than solvent-containing bilayers (Table 1).

Membrane conductance of decane-MGDG bilayers

The I-V relationship of membranes formed in different concentrations of potassium chloride as well as in other alkali metal chlorides showed deviations from Ohm's law. Curves obtained with aqueous phase compositions of 1 mM, 150 mM, and 1 M KCl are shown in Fig. 1 *A*. The central portion of the curves (±60 mV) was essentially ohmic (insert Fig. 1 *B*). At high electrical potentials, the I-V relationship is nonlinear. In general, the breakdown voltage for membranes in solutions of chloride salts of hydrogen, lithium, sodium, potassium, magnesium, and calcium was in the range between 150 and 200 mV. Using our value of 5 nm for the membrane thickness, the dielectric strength of gactolipids at the breakdown voltage was about 3×10^5 V/cm.

The dependence of decane-MGDG bilayer conductance on the cation present in the aqueous phase was examined. The current-voltage relationship obtained with membranes generated in 0.5 M solutions of chloride salts of lithium, potassium, and sodium are shown in Fig. 1 *B*. The specific membrane conductances calculated from the regression of the linear portion of the I-V curve were 4.0×10^{-8} S cm^{-2}, 4.2×10^{-8} S cm^{-2}, and 6.3×10^{-8} S cm^{-2} for KCl, NaCl, and

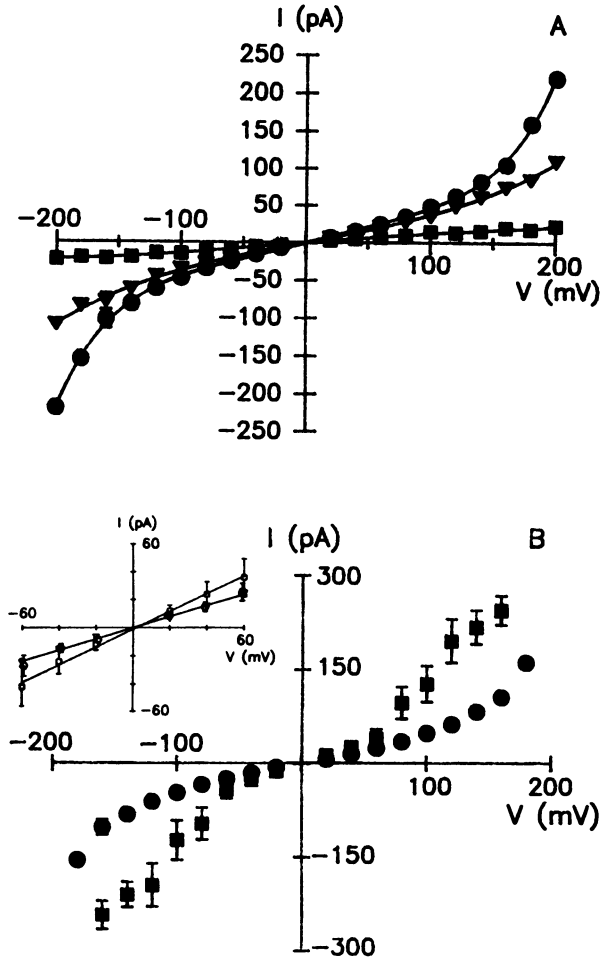

FIGURE 1 (*A*) The I-V relationship of planar lipid membranes from decane-MGDG for three different concentrations of KCl: 1 mM (■), 150 mM (▲), 1 M (●), and 10 mM HEPES buffer pH 7.2. (*B*) The I-V relationship of decane-MGDG bilayers in 0.5 M KCl and NaCl (●) or 0.5 M LiCl (■) and 10 mM HEPES buffer pH 7.2. (*Insert*) plot of the ohmic part of the I-V curves in 0.5 M KCl (○), 0.5 M NaCl (△), and 0.5 M LiCl (□) buffered with 10 mM HEPES pH 7.2.

LiCl, respectively (Fig. 1 *B*, *inset*), and were not statistically different. Only Li^+ increased significantly the specific conductance at higher potentials (>60 mV) (Fig. 1 *B*).

BLM formed from decane-MGDG were more permeable to ions than decane-lecithin bilayers (Fig. 2). The conductance of MGDG and soybean lecithin was independent of the KCl concentration over the range 1 mM to 0.5 M. MGDG BLM in 0.15 M KCl had specific conductance of $3.7 \pm 0.4 \times 10^{-8}$ S cm^{-2} ($n = 10$). The specific conductance of lecithin bilayer membrane in 0.1 M KCl was found to be $1.4 \pm 0.5 \times 10^{-9}$ S cm^{-2} ($n = 7$). This value is 26-fold lower than that of MGDG bilayers.

Because there is a free magnesium chloride concentration ranging between 1 and 3 mM inside the chloroplast in the dark (Portis, 1981), we decided to examine the effect of the addition of this cation to the *cis* compartment on the specific conductance of MGDG bilayers formed in 100 mM KCl (pH 7.2). In the range of concentration studied (1–80 mM), the

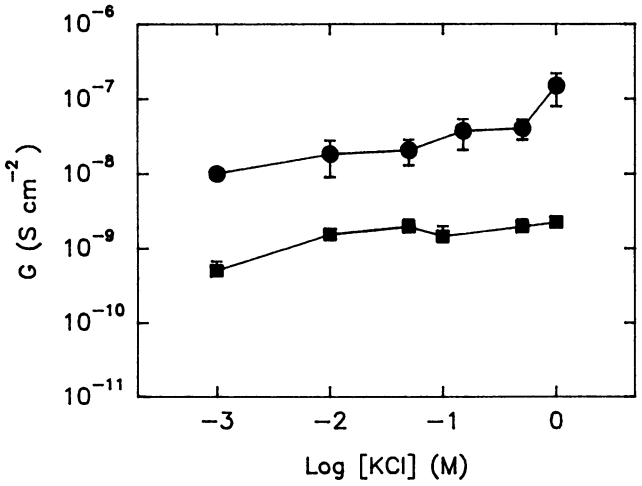

FIGURE 2 Specific conductance of decane-MGDG (●) and decane-soybean lecithin (■) as a function of the KCl concentrations of the bathing medium. The buffer used was 10 mM HEPES (pH 7.2). All conductances were determined with a potential of 60 mV.

addition of magnesium had no significant effects on specific bilayer conductance (results not shown). Moreover, the specific conductance of MGDG bilayers measured in unbuffered 10 mM magnesium or calcium chloride were of the same order of magnitude as that measured in potassium chloride: $8.3 \pm 0.8 \times 10^{-9}$ S cm^{-2} ($n = 3$) for $MgCl_2$, $4.6 \pm 0.9 \times 10^{-9}$ S cm^{-2} ($n = 5$) for $CaCl_2$, and $1.9 \pm 0.8 \times 10^{-8}$ S cm^{-2} ($n = 6$) for KCl.

The specific resistance of the decane-MGDG and decane-lecithin bilayers as a function of the pH was measured in the presence of 100 mM KCl. The buffer solutions used were 10 mM citric acid (pH 2), formic acid (pH 3–4.8), MES (pH 5.2–6), HEPES (pH 7.2), and TRIS (pH 8–9.3). Our measurements revealed a weak dependence of the specific resistance of lecithin BLM on pH (Fig. 3). The MGDG bilayers were not pH-sensitive.

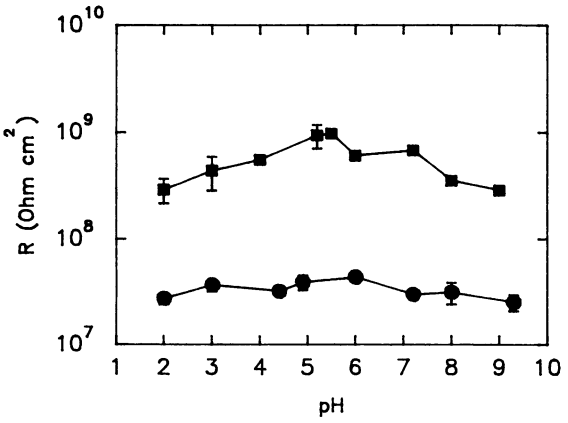

FIGURE 3 Specific resistance of decane-MGDG (●) and decane-soybean lecithin membranes (■) as a function of pH in 100 mM KCl. The pH was controlled by 10 mM TRIS, HEPES, MES, formic acid, or citric acid depending on the pH range used. All resistances were determined with a potential of 60 mV.

Membrane conductance of thylakoid lipid mixtures

Several experiments were performed with a mixture of thylakoid lipids in decane chosen to mimic the lipid composition of thylakoids in vivo (Fig. 4 *A*). The specific conductance of decane-containing MGDG/DGDG/SQDG/PG bilayers (2:1:0.5:0.5, w/w) in 150 mM KCl was $G_m = 1.1 \pm 0.1 \times 10^{-8}$ S cm^{-2} ($n = 5$). Unfortunately, it was not possible to obtain the contribution of DGDG to the membrane permeability because this compound alone was not entirely dissolved in n-decane at room temperature. A BLM was made from a binary mixture of MGDG/lecithin (1:1, w/w) in decane to verify that the conductance of the phospholipid would be changed by the presence of MGDG. The I-V curve of the BLM was increased significantly in the presence of MGDG (Fig. 4 *B*). The specific conductance measured in 100 mM KCl was found to be $G_m = 3 \pm 0.3 \times 10^{-9}$ S cm^{-2} ($n = 4$), which resulted in a twofold higher specific conductance than lecithin. The specific conductance of the quaternary mixture of thylakoid lipids (with or free of solvent) was also inde-

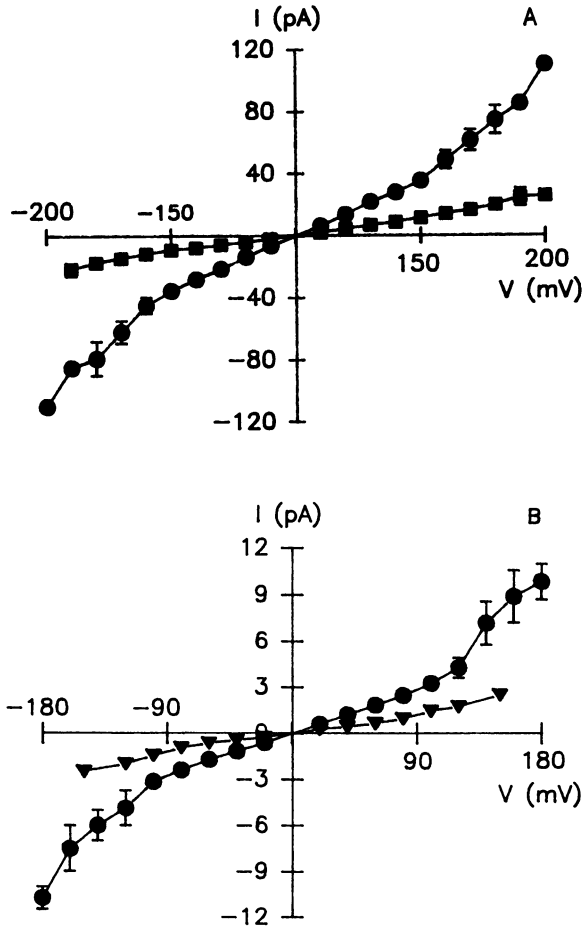

FIGURE 4 (*A*) The I-V relationship of decane-MGDG (●) and decane-MGDG/DGDG/SQDG/PG (■) bilayers in 150 mM KCl, 10 mM HEPES (pH 7.2). The ratio (w/w) for the quaternary mixture was 2:1:0.5:0.5. (*B*) The I-V relationship of decane-MGDG/lecithin (1:1, w/w) (■) and soybean lecithin (▲) bilayers in 100 mM KCl, 10 mM HEPES (pH 7.2).

pendent of the KCl concentration (Fig. 5). For the decane-containing bilayers, we changed slightly the experimental setup by using a styrene-copolymer cup with a hole of 200 μm in diameter instead of the teflon beaker. The specific conductance of the decane bilayers shown in Fig. 5 are different as compared with that obtained with the teflon beaker (Fig. 4 *a*). The specific conductance of the decane-quaternary mixture over the range 1 mM to 1 M was about fivefold higher than that of phospholipids, i.e., in 150 mM KCl: $G_m = 4.4 \pm 0.7 \times 10^{-8}$ S cm^{-2} ($n = 3$) and $G_m = 1.1 \pm 0.1 \times 10^{-8}$ S cm^{-2} ($n = 3$), respectively. When we formed solvent-free planar bilayers, the specific conductance of the galactolipids was about tenfold larger than that of lecithin. The current-voltage relationship obtained for solvent-free bilayers of MGDG/DGDG/SQDG/PG in 10 mM KCl is shown in Fig. 6. The specific conductances calculated in 150 mM KCl were $6.0 \pm 1.1 \times 10^{-8}$ S cm^{-2} ($n = 3$) and 7×10^{-9} S cm^{-2} ($n = 3$) for the quaternary mixture and lecithin bilayers, respectively.

Despite the absence of solvent inside the membrane, we observed that the experimental I-V curve was not ohmic at high electrical potential differences (>60 mV). This is similar to what is observed with decane-lipid bilayers. For both solvent-free and decane bilayers, the ratio of the specific conductance at a specified voltage to that at 20 mV plotted against the applied potential showed that the conductance-voltage relationships were nonlinear (Fig. 7). The dotted lines are theoretical curves simulated using the following equation for a trapezoidal energy barrier (Hall et al., 1973, Krishnamoorty and Hinkle, 1984; Gutknecht, 1987)

$$\frac{G}{G_{20}} = \frac{b \sinh(FV/2RT)}{\sinh(b\,FV/2RT)}, \qquad (1)$$

Where F, R, and T have their usual meanings, and b is the fraction of the membrane thickness spanned by the minor base of the trapezoid. The model fits the majority of the data, with b ranging from 0.7 to 0.9. When $b = 1$ the trapezoïdal energy barrier model reduced to Ohm's law. The solid line is the prediction of the following equation, a single barrier Eyring model

$$\frac{G}{G_{20}} = \frac{RT}{F} \sinh\left(\frac{FV}{2RT}\right). \qquad (2)$$

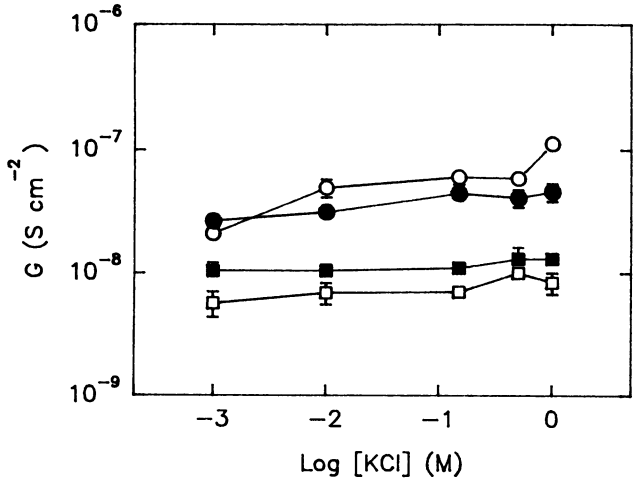

FIGURE 5 Specific conductance of solvent-free (○, □) and decane-bilayers (●, ■) from MGDG/DGDG/SQDG/PG (○, ●) and soybean lecithin (□, ■) as a function of the KCl concentrations of the bathing medium. The buffer used was 10 mM HEPES (pH 7.2). All conductances were determined with a potential of 60 mV.

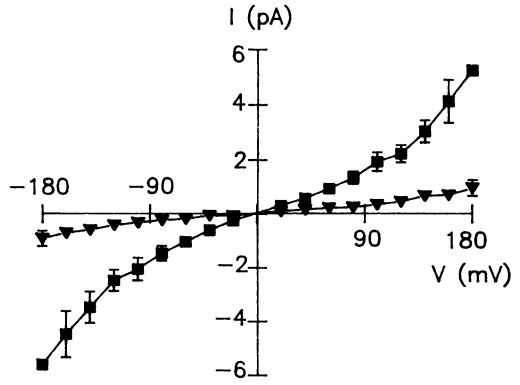

FIGURE 6 The I-V relationship of solvent-free MGDG/DGDG/SQDG/PG (■) and soybean lecithin (▲) bilayers in 10 mM KCl buffered with 10 mM Hepes (pH 7.2).

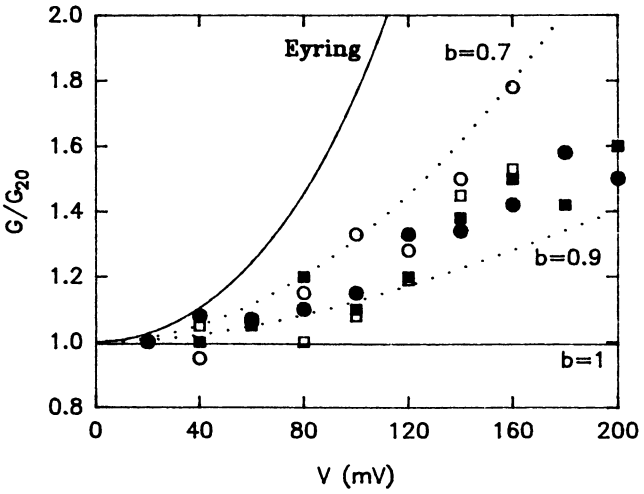

FIGURE 7 The normalized conductance-voltage relationship of solvent-free (○, □) and decane-bilayers (●, ■) from MGDG/DGDG/SQDG/PG mixtures. The $G_{H/OH}$ (□, ■) was measured in symmetrical pH 7.75, and the total membrane conductance G_m (○, ●) was measured in symmetrical 150 mM KCl, 10 mM Hepes (pH 7.2). The dotted lines were calculated from Eq. 1 with b = the fraction of the membrane spanned by the minor base of the trapezoid. Each point represents the mean of one to six experiments.

Proton and hydroxyl permeability

The H^+/OH^- diffusion potentials, V_m, produced by two mixtures of weakly basic and acidic buffers over the physiological pH ranges of pH 6.0–6.5 and 7.4–8.1 are shown in Table 2. From these values and the Nernst equilibrium potential,

TABLE 2 Membrane diffusion potentials (V_m), H/OH transference numbers ($T_{H/OH}$), H/OH specific conductance ($G_{H/OH}$), and proton permeability coefficients (P_H) in decane-lipid bilayers and in solvent-free bilayers formed from osmotically balanced buffer mixtures producing a pH 6.0/6.5 gradient (MES/BIS-TRIS) or a pH 7.4/8.1 gradient (HEPES/TRIS)*

	Decane-MGDG pH 6.0/6.5	Decane-MGDG pH 7.4/8.1	Decane-MGDG/ DGDG/SQDG/PG pH 7.4/8.1	Solvent-free MGDG/ DGDG/SQDG/PG pH 7.4/8.1
G_m (S cm^{-2})	7.9 ± 0.7 10^{-9} (10)	6.0 ± 0.5 10^{-9} (7)	7.6 ± 0.3 10^{-9} (5)	1.2 ± 0.5 10^{-8} (4)
$E_{H/OH}$ (mV)	−29.5	−41.3	−41.3	−41.3
V_m (mV)	−17.3 ± 0.4 (4)	−26.0 ± 1.3 (7)	−28.0 ± 1.9 (4)	−25.3 ± 1.3 (3)
$T_{H/OH}$	0.59 ± 0.01 (4)	0.63 ± 0.03 (7)	0.68 ± 0.05 (4)	0.61 ± 0.03 (3)
$G_{H/OH}$ (S cm^{-2})	4.6 ± 0.1 10^{-9} (4)	3.8 ± 0.2 10^{-9} (7)	5.1 ± 0.4 10^{-9} (4)	7.4 ± 0.3 10^{-9} (3)
P_H (cm s^{-1})	2.2 ± 0.1 10^{-6} (4)	5.7 ± 0.3 10^{-5} (7)	7.7 ± 0.5 10^{-5} (4)	1.1 ± 0.1 10^{-4} (3)

* The numbers in parentheses indicate the number of replicates; the membrane conductance (G_m) was measured in a symmetrical pH = 6.25 or pH = 7.75.

$E_{H/OH}$, the transference number of H^+/OH^- can be calculated

$$T_{H/OH} = \frac{V_m}{E_{H/OH}}. \quad (3)$$

In the two pH ranges studied, the decane-MGDG membrane is moderately H^+-selective ($T_{H/OH}$ = 0.59 and 0.63). In the pH 7.4–8.1 gradient, the solvent-free and decane-containing MGDG/DGDG/SQDG/PG bilayers are also H^+-selective ($T_{H/OH}$ = 0.68 and 0.61). The H/OH conductance was calculated from the following relation

$$G_{H/OH} = (T_{H/OH})G_m, \quad (4)$$

where G_m is the total steady-state membrane conductance measured in symmetrical buffer concentrations (pH = 7.75 and 6.25). G_m was also measured in the presence of the two pH gradients but did not change significantly. This result is similar to that obtained with phospholipids (Gutknecht, 1984). We have calculated the permeability coefficient for proton, P_H, using the following relation and assuming that H^+ is responsible for $G_{H/OH}$

$$P_H = \frac{(G_{H/OH})RT}{F^2C_H}. \quad (5)$$

The permeability coefficient for proton has been calculated at pH = 6.25 or 7.75 (Table 2).

H/OH current-voltage curves of solvent-free and decane-containing quaternary mixtures of lipids were nonlinear at high voltages (results not shown). The normalized $G_{H/OH}$ plotted against the applied voltage are shown in Fig. 7.

Potassium and chloride permeability

A salt solution of 100 mM KCl (*cis* side) separated by the bilayer from a solution of 10 mM KCl (*trans* side) gave similar cationic diffusion potential across three kinds of BLM (Table 3: decane-containing MGDG, solvent-free, and decane-containing MGDG/DGDG/SQDG/PG bilayers), indicating that these membranes are selectively permeable for cations. Control experiments showed that measured diffusion potentials in a tenfold KCl gradient after the breakage of the lipid bilayer are in agreement with the theoretical value of 1 mV. Thus, the measured diffusion potentials were not due to junction potentials. A typical I-V curve recorded in the presence and in the absence of a KCl gradient is shown in Fig. 8. The reverse potential measured was more positive than the equilibrium potential, thus indicating that decane-MGDG membranes are not perfectly selective to cations. Using the data of Tables 2 and 3, we can calculate that the specific conductance $G_{H/OH}$ accounts for only 10% of the total BLM conductance measured in the presence of KCl. Therefore, assuming that $G_{H/OH}$ can be neglected from the total

TABLE 3 Membrane diffusion potentials (V_m), ionic transference numbers (T_K or T_H), and permeability coefficients (P_K, P_H, and P_{Cl}) in decane-lipid bilayers and in solvent-free bilayers formed in KCl or in HCl gradients*

Concentration ratio 100/10 mM KCl	Decane-MGDG	Decane-MGDG/ DGDG/SQDG/PG	Solvent-free MGDG/ DGDG/SQDG/PG
G_m (S cm^{-2})	3.7 ± 0.4 10^{-8} (9)	4.4 ± 0.7 10^{-8} (3)	6 ± 1.1 10^{-8} (3)
V (mV)	−29.7 ± 1.5 (7)	−34.4 ± 6.8 (10)	−34 ± 2.5 (5)
T_K	0.75 ± 0.01 (7)	0.79 ± 0.06 (10)	0.78 ± 0.02 (5)
P_K	4.9 ± 0.1 10^{-11} (7)	6.2 ± 0.5 10^{-11} (10)	8.3 ± 0.2 10^{-11} (5)
P_{Cl}	1.6 ± 0.1 10^{-11} (7)	1.6 ± 0.5 10^{-11} (10)	2.3 ± 0.2 10^{-11} (5)
Concentration ratio 1/0.1 mM HCl	**Decane-MGDG**	**Decane-MGDG/ DGDG/SQDG/PG**	**Solvent-free MGDG/ DGDG/SQDG/PG**
G_m (S cm^{-2})	5.2 ± 0.7 10^{-9} (3)	3.9 ± 0.6 10^{-8} (3)	5.9 ± 0.9 10^{-8} (4)
V (mV)	−75.7 ± 1.2 (3)	−77 (1)	−104 (2)
T_H	0.82 ± 0.01 (3)	0.83 (1)	0.94 (2)
P_H	1.1 ± 0.1 10^{-9} (3)	8.6 10^{-9} (1)	1.5 10^{-8} (2)
P_{Cl}	2.4 ± 0.1 10^{-10} (3)	1.8 10^{-9} (1)	9.4 10^{-10} (2)

* The numbers in parentheses indicate the number of replicates. The membrane conductance (G_m) was measured in a symmetrical 150 mM KCl or 1 mM HCl.

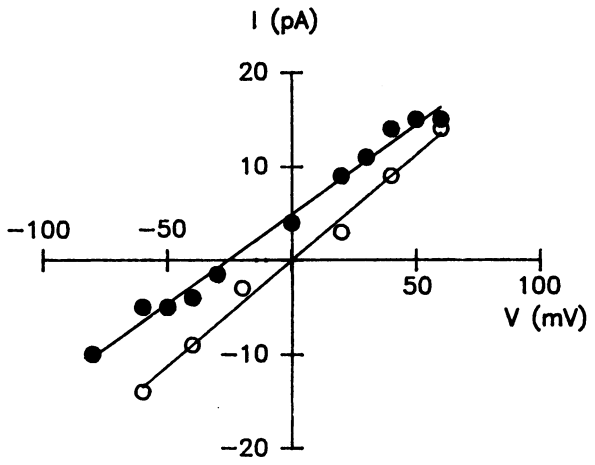

FIGURE 8 The I-V relationship of typical MGDG bilayer under asymmetrical (●) KCl concentrations (100 mM KCl in the *cis* side and 10 mM KCl in the *trans* side buffered with 10 mM HEPES, pH 7.2) and symetrical KCl concentration of 100 mM (○).

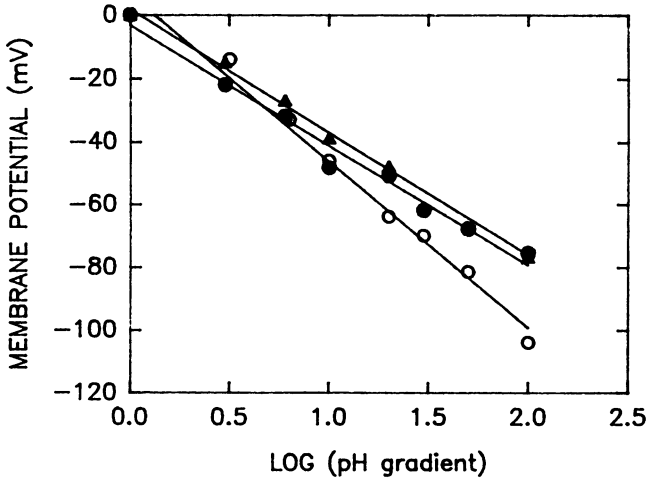

FIGURE 9 Diffusion potentials generated by HCl gradients through solvent-free (○, △) and decane bilayers (●, ▲) from MGDG (△, ▲) and MGDG/DGDG/SQDG/PG (○, ●). Membranes were formed in symetrical 0.1 mM HCl. Each point represents one to three experiments in which 1 M HCl was added to *cis* compartment.

membrane conductance, then ionic transference numbers for potassium can be calculated from the relations

$$V_m = (2T_K - 1)E_K. \quad (6)$$

The potassium transference numbers, T_K, defined as G_K/G_m, where G_m is the total conductance in KCl, ranged from 0.75 to 0.79 in the three types of planar lipid membranes (Table 3).

HCl permeability

The diffusion potentials were measured as a function of the HCl gradient across the membrane. Our attempts to measure the potentials in the same gradient as that used with KCl (10^{-1}–10^{-2} M) were unsuccessful because the bilayers were unstable. Therefore, we used a concentration of HCl of 10^{-4} M in each chamber. The HCl concentration in the *cis* side was then increased stepwise from 10^{-4} M to a final concentration of 10^{-2} M. In the presence of an HCl gradient decane-containing MGDG, decane-containing MGDG/DGDG/SQDG/PG, and solvent-free MGDG/DGDG/SQDG/PG bilayers exhibited proton selectivity (Fig. 9). The experimental potentials depart from the diffusion potential calculated for an ideal proton selective membrane (59 mV/pH unit). Control experiments with salt bridges at different pH were performed to show that differences in junction potential between the bridge and the solution of the *cis* compartment can be neglected. The ionic transference numbers of solvent-free and decane-containing bilayers were estimated from the equilibrium potential in a 100-fold concentration gradient across the membrane. The membrane potential was a linear fonction of the logarithm of the pH gradient indicating that the potentials measured were diffusion potentials. The values of T_H of decane-bilayers and solvent-free bilayers were significantly different, with T_H = 0.82 and 0.9, respectively. From the total membrane conductance in 1 mM HCl, we obtained P_H of one order of magnitude higher than P_{Cl} in the same experimental conditions (Table 3).

DISCUSSION

Effects of n-decane on bilayers properties

The MGDG molecule resembles a cone in shape, whereas DGDG, PG, and SQDG molecules show overall cylindrical shapes. The consequence is that the MGDG reconstituted in aqueous solutions is unable to form a bilayer membrane, whereas the others readily pack into lamellar structures. However, Graziani and Livne (1972) obtained a BLM formed from decane-MGDG and calculated its water permeability of 7.3×10^{-5} cm s^{-1}. Our measurements for the specific capacitance of decane-MGDG confirm the bilayer nature of the lipid film (Table 1). The apparent thickness of the hydrophobic core of this bilayer is the same as that of a decane-lecithin bilayer (Table 1). Specific capacitances of decane-MGDG bilayers are lower than those of natural thylakoid membrane, 1.2 ± 0.3 μF cm^{-2} (Farkas et al., 1984) because some decane was present in the bilayers. It has been shown previously that residual decane between 0.35 and 0.45 v/v PC remains in the bilayer after the thinning (Pagano et al., 1972; Tien, 1973). The folding of two monolayers of MGDG did not result in the formation of a bilayer despite an initial rise of the capacitance (excepted in about 5% of the trials, see Table 1). This means that decane greatly enhances the stability of the planar configuration of MGDG bilayers. This solvent, not present in biomembranes, does not affect the transference number and permeability coefficient for K^+, Cl^-, and H^+/OH^- (Tables 2 and 3). In one case, however, the presence of decane decreased slightly T_H in BLM formed in HCl gradients when the logarithm of the pH gradient was >1 (Table 3 and Fig. 9). The addition of other thylakoid lipids (SQDG, DGDG, and PG) to MGDG with similar ratios as that found in the native membrane promotes the formation of solvent-free bilayers from a mixture containing high concentration of MGDG. The specific capacitance of solvent-free bilayers formed from a quaternary mixture was close to

that of thylakoids. Although the so-called solvent-free membranes are formed with hexane and squalene, we can assume that they contain negligible amounts of solvent because squalene is insoluble in phospholipids and hexane has a limited solubility in water and is highly volatile. If a small amount of squalene remains in the bilayer, it probably appears as microlenses (Benz et al., 1975; White, 1976; Simon et al., 1977; Reyes and Latorre, 1978). Furthermore, the bilayer thickness of the BLM is very close to that deduced from x-ray diffraction, implying that essentially solvent-free membranes are formed from monolayers.

The normalized conductance of solvent-free and decane bilayers was increased by the membrane potential (Fig. 7). It has been shown that the electric field creates a compressive force that leads to a decrease in the amount of solvent in decane-containing membranes and that further thins the film (Benz et al., 1975). But the decrease of thickness cannot explain the shape of the I-V curve. Indeed, our results show that the I-V curves of solvent-free bilayers were also nonlinear. Although the transport process of ions through lipid bilayers has been based on the potential dependence of the membrane conductance, we applied two different models to fit our data. Our results of Fig. 7 show that ion conduction cannot be explained by the Eyring rate theory if we assume that the translocation of ions across the membrane occurs as a jump across a single activation energy barrier. Instead, the trapezoidal energy barrier model could explain the moderate (b = 0.7–0.9) voltage dependence of the ion transport to the membrane potential.

Comparison between decane-BLM from phospholipids and MGDG

The decane-MGDG bilayer does not select between cations. Only at transmembrane potentials higher than 60 mV is lithium more permeable as compared with others cations (Fig. 1 *b*). This result is consistent with the decrease of the specific resistance of egg PC in the presence of LiCl (Miyamato and Thompson, 1967). The membranes formed from decane-MGDG showed a 26-fold higher conductance than that of BLM formed from decane-phospholipids (Fig. 2). The specific conductances of decane-MGDG, lecithin, and MGDG/DGDG/SQDG/PG were independent of the KCl concentration over the range 1 mM to 0.5 M as reported with other membranes (Andreoli et al., 1967; Gutknecht and Tosteson, 1970). It was found that the conductance of the decane-MGDG bilayer membrane did not change significantly when divalent cations replaced or were added to KCl. This result was expected because MGDG is a neutral lipid. Indeed, with PS bilayers the increase of resistance was explained by chelation of divalents on the negatively charged head groups (Miyamato and Thompson, 1967; Ohki, 1969a). Our investigation of the conductance as a function of the pH revealed a dependence of decane-lecithin BLM on pH. The variation of resistance of phospholipids, less than one order of magnitude, was statistically significant. This can be attributed to the dissociation of polar head groups of the components of soybean lecithin, which may produce structural changes in the lipids. The pH range of the maximum of resistance (around pH 5) may correspond to an isoelectric point of the mixture of phospholipids where the repulsive forces would be the weakest. As suggested previously (Ohki, 1969a), the consequence is the thickening of the bilayer and the increase of the resistance. However, the correlation between the variation of capacitance and resistance with pH measured for egg PC, diphytanoyl PC, and PS is not clear (Ohki, 1969a, b; Redwood et al., 1971). Our results support the hypothesis according to which polar head groups are indispensable to observe the pH effect, because the resistance of the neutral lipid MGDG did not change with pH.

Stabilization of MGDG by cylindrical lipids

Fig. 5 showed that the specific conductance of solvent-free and decane-containing bilayers formed from the quaternary mixture of thylakoid lipids is about five- to tenfold higher than that of the phospholipid bilayers. The specific conductance of the decane-quaternary mixture was 3 times lower than that of the decane-containing MGDG bilayer (Fig. 4 *a*). Furthermore, when MGDG is added to phospholipids, the specific conductance increased twofold (Fig. 4 *b*). These results support the hypothesis according to which MGDG contributes significantly to the high conductance of the quaternary mixture of lipids.

Cylindrical lipids are required to form solvent-free BLM with MGDG. The ratio used, similar to that observed in vivo, was critical. Indeed, BLM was never formed when the fraction of MGDG was increased. Our results suggest that the cylindrical lipids DGDG, SQDG, and PG can structure the cone-shaped MGDG and promote the formation of a planar bilayer. Thus, our results do not agree with the hypothesis of Gounaris et al. (1983), according to whom the bilayer structure of the thylakoid lipids would be stabilize by proteins.

Role of galactolipids on the passive proton permeabilty of thylakoids

Table 2 shows evidence that the decane-containing bilayers of MGDG and MGDG/DGDG/SQDG/PG and solvent-free MGDG/DGDG/SQDG/PG bilayers are proton-selective. At neutral to alkaline pH, PC membranes were also moderately H^+-selective with $T_{H/OH}$ from 0.4 to 0.8 (Gutknecht, 1984). The proton-hydroxyl permeability was an area of considerable controversy as there are as many as six orders of magnitude difference between the permeability coefficients in the literature (Deamer and Nichols, 1983). Gutknecht (1984) has observed that the proton conductance is relatively constant with pH, so the H/OH permeabilities are highly pH-dependent (see Eq. 5). This could explain the disparity of the values published. For this reason, we chose physiological conditions in the pH range 6–8.

The magnitude of the H^+/OH^- conductance is not high (Table 2). At pH 7, decane-diphytanoyl PC and decane-PE bilayers have $G_{H/OH}$ from 1 to 6 $\times$ 10^{-9} S cm^{-2} (Gutknecht,

1984, 1987). Because of the low proton concentration, we calculated that planar bilayers from thylakoid lipids are extremely permeable to proton (Table 2). This is consistent with studies on liposomes and planar membranes formed from phospholipids, which showed high proton-hydroxyl permeabilities between 10^{-5} to 10^{-3} at neutral pH ranges (Nichols and Deamer, 1980; Deamer and Nichols, 1983; Gutknecht, 1984; Deamer, 1987). Until now, the only values for plant lipids were reported on liposomes from mixed phospholipids with P_H ranging from 1.9×10^{-5} cm s^{-1} to 3×10^{-3} cm s^{-1} (Rossignol et al., 1982; Krishnamoorthy and Hinkle, 1984; Grzesiek and Dencher, 1986). These values suggest that the mechanism of proton permeability is different from that of other ions. Two mechanisms have been proposed: a passive pathway can occur via low amounts of weakly acid contaminants, such as fatty acids, that act as protonophores (Gutknecht and Walter, 1981a; Gutknecht, 1984, 1987) or hydrogen jumps along transient hydrogen-bonded chains of water (Nichols and Deamer, 1980; Nagle and Nagle, 1983; Nagle, 1987; Cafiso and Hubbel, 1987; Deamer, 1987). There is no direct evidence for such mechanisms. The weak acid hypothesis is supported by experiments on planar lipid membranes. The addition of phloretin, which reduces the membrane dipole potential, was found to decrease about tenfold the H/OH conductance, so Gutknecht (1984) expected that H/OH flux is performed by an anionic carrier. The tenfold decrease of $G_{H/OH}$ by bovine serum albumin (BSA), which binds fatty acids, supports the idea that the trace contaminants are fatty acids that act as proton carriers (Gutknecht, 1987). Furthermore, the addition of fatty acids to the bilayer increases $G_{H/OH}$. In contrast, the weak acid hypothesis cannot explain a basal $G_{H/OH}$ that remains at acidic pH and after the addition of inhibitors (BSA, phloretin). This basal conductance appears still some several orders of magnitude higher than that for K^+ and does not seem to be explained by hydrated defects in the bilayers because $G_{H/OH}$ is sensitive to changes of the dielectric constant and internal dipole potential. In liposomes, the results are different from those observed in BLM. The weak acid hypothesis is not valid because the addition of phloretin and fatty acids has no effects on the $G_{H/OH}$ (Cafiso and Hubbel, 1987). Moreover, BSA does not inhibit proton flux across liposomes (Deamer, 1987). On the other hand, the alternative view, that protons jump along transient hydrogen-bonded strands of water molecules, has indirect experimental support. Gramicidin A has been used as a model of a single file of water molecules to provide experimental support to the H^+ jump hypothesis. It is at least four orders of magnitude more permeable to proton than to potassium (Deamer, 1987). However, its conductance is highly pH-dependent, insensitive to chlorodecane, and reduced in D_2O. This is opposite to what has been measured in BLM and liposomes in the absence of gramicidin (Gutknecht, 1987; Deamer, 1987). According to Nagle (1987) and Deamer (1987), a strand of water through the bilayer could be formed by the transient association of half chains of water from opposite sides of the membrane. In summary, the nature of the molecular mechanism for proton permeability is unknown. The weak acid contaminants may contribute to a part of the proton flux across planar bilayers but cannot account for the proton conductance in liposomes.

Our data shown in Fig. 7 indicate that the increase of normalized $G_{H/OH}$ by voltage can be fitted by the trapezoidal energy barrier model. The fraction of the membrane spanned by the minor base of the trapezoid was similar with that estimated previously from $G_{H/OH}$ measurements on phospholipids (Gutknecht, 1987: b = 0.7–0.9; Krishnamoorty and Hinkle, 1984: b = 0.75), but the voltage dependence was lower than that measured with protonophores (b = 0.55–0.65).

The proton permeability of nonphosphorylating thylakoid membranes of 2×10^{-5} cm s^{-1} (Schönfeld and Schickler, 1984) is close to that found for planar bilayers from thylakoid lipids. We suggest that H^+ diffusion through thylakoid lipid matrix can account for unspecific proton leakage through the native membrane. The chemiosmotic theory implies that the proton gradient is maintained against a leak down an electrochemical gradient. Despite the high intrinsic proton permeability coefficient measured in the thylakoid lipid, the bilayer is a significant barrier to protons because the H^+ concentration inside the thylakoid is so small compared to physiological concentrations of potassium and chloride ions. The passive proton flux outside thylakoid membranes is negligible and cannot short-circuit the proton motive force of the coupling membrane.

Does the membrane dipole potential control the ion selectivity?

Flewelling and Hubbel (1986) have developed a model to explain the selectivity of lipid bilayers to hydrophobic anions and cations. The specific conductance for the negative tetraphenylborate was known to be some 10^4-fold larger than the positive tetraphenylphosphonium. According to their model, the energy profile of the ion permeation through the lipid membrane is the sum of three terms (the Born-image energy, the dipole electrical energy, and the hydrophobic energy). In their model, the dipole potential of ester groups of lipid carbonyls is believed to make the largest contribution to the membrane dipole potential (about 240 mV positive inside). It results in a low energy barrier for hydrophobic anions as compared to hydrophobic cations. Changing ion radius and assuming no hydrophobic energy contributions, they extended the energy profile model to small inorganic ions and concluded that the energy barrier for anions (Cl^-, Br^-) is lower than that for cations (K^+, Na^+). Nevertheless, the permeability barrier for small inorganic ions is still very large as compared with hydrophobic ions. This generalization to inorganic ions is not supported by our experimental results. Indeed, (i) theoretical ion permeability coefficients calculated from the free energy barrier of this model would be several orders of magnitude lower than the values in Table 3 (Hauser et al., 1973), and (ii) planar lipid bilayers formed from thylakoid lipids showed a selective permeability to K^+ over Cl^-. Moreover, it has been shown previously that planar

membranes from zwitterionic phospholipids (PC and PE) and uncharged lipid (DGluDG) showed slight cation selectivity with T_K from 0.55 to 0.65, and negatively charged lipids (PS, PG, and diPG) were highly selective with T_K from 0.7 to 0.95 (Miyamato and Thompson, 1967; Andreoli et al., 1967; Hopfer et al., 1970; Toyoshima and Thompson, 1975; Gutknecht and Walker, 1981b). Transference numbers of the quaternary mixture of thylakoid lipids, which contains negative lipids (SQDG and PG), are in the range of values reported for other negative lipids.

From tracer flux experiments on liposomes and spherical BLM, it has been known for some time that small anions exhibit permeability coefficients for phospholipid bilayers some two to three orders of magnitude larger than for K^+ or Na^+ (Pagano and Thompson, 1968; Hauser et al., 1973; Toyoshima and Thompson, 1975). This is not the case for liposomes formed with DGDG from chloroplast (Webb and Green, 1989). P_{Cl} measured by isotopic fluxes was about two to three orders of magnitude higher than predicted from electrical measurements. To explain the anionic selectivity of phospholipid liposomes and spherical BLM, it was suggested that the chloride flux could be separated into (i) a large net flux of neutral molecular HCl inside the membrane, whereas the chloride transfer across the interface should be catalyzed by the choline groups of phospholipids (Toyoshima and Thompson, 1975; Robertson and Thompson, 1977) and (ii) a small transfer inside the bilayer of charged Cl^-. Our investigation of HCl transport through thylakoid lipids did not show any electroneutral process, perhaps because no choline groups are present in thylakoid lipids (Dorne et al., 1990). The planar membranes formed from thylakoid lipids were highly selective to H^+ (Fig. 9 and Table 3), with transference numbers similar to those measured with BLM of PG, PE, DGluDG, and *E. coli* lipids ranging from 0.79 to 0.95 (Hopfer et al., 1970; Tien, 1974). In contrast, Gutknecht and Walter (1981a) found that H^+ was transported as neutral HCl molecules across phospholipid BLM, but the HCl flux was several orders of magnitude smaller than that measured in liposomes.

The surface potentials measured on monolayers of DGDG (310 mV), MGDG (320 mV), and SQDG (260 mV) are similar to those of phospholipids (Oldani et al., 1975). Therefore, the membrane dipole potential of a mixture of galactolipids is probably positive inside, which is in contradiction with the cationic selectivity. In the thylakoid membrane, the contribution of the negative surface charge of SQDG and PG headgroups coud be prevalent for ion permeation. An alternative pathway for ions is that they enter and move in the galactolipid bilayers as hydrated species in rare transient defects, which are postulated to form spontaneously (Gutknecht, 1984; Deamer, 1987).

Role of galactolipids on the passive K^+ and Cl^- permeability of thylakoids

The K^+ and Cl^- permeability coefficients of decane-containing MGDG bilayers and solvent-free thylakoid lipid mixtures calculated in Table 3, fall in the range of 10^{-10} to 10^{-12} cm s^{-1} measured electrically in planar phospholipid bilayers (Toyoshima and Thompson, 1975; Gutknecht and Walter, 1981b). Reported values for the thylakoid membranes were three orders of magnitude higher, i.e., $P_{Cl} = 2 \times 10^{-8}$ cm s^{-1} and $P_K = 4 \times 10^{-8}$ cm s^{-1} (Barber, 1972; Vredenberg, 1976; Van Kooten et al., 1986). Furthermore, Junge and Jackson (1982) have calculated the passive ionic conductance of thylakoids (2×10^{-5} S cm^{-2}) from the decay of the electrochromic pigment absorption shift after a flash of light. A similar value of 8×10^{-5} S cm^{-2} was calculated by Vredenberg (1976) from experimental data taken in the literature. We observe a large disparity of conductance between the biological membrane and that of thylakoid lipid planar bilayers: the conductance of planar bilayers made from MGDG and MGDG/DGDG/SQDG/PG is some three orders of magnitude less than passive conductance of thylakoids. Because planar lipid bilayers from MGDG and quaternary mixture of MGDG/DGDG/SQDG/PG are relatively impermeable to K^+ and Cl^- ions, the passive permeability of thylakoid cannot be determined significantly by the galactolipids, as suggested by Webb end Green (1989). The ionic transport through the thylakoid involves membrane constituents other than lipids. For instance, chloride selective and potassium selective channels have been detected in thylakoids (Scönknecht et al., 1988; Tester and Blatt, 1989; Enz et al., 1993). They should be responsible for anion and cation fluxes, which compensate for the net H^+ uptake into the intrathylakoid space.

In conclusion, our study shows that the addition of cylindrical thylakoid lipids to MGDG is sufficient to promote a planar bilayer configuration without the presence of decane. The ion transport through the planar membranes was not affected by residual decane in the lipid film. We show that the galactolipid bilayers are cation-selective but relatively impermeable to most ions. However, the specific conductance of planar bilayers formed from thylakoid lipids is about one order of magnitude larger than that of lecithin bilayers. Despite this difference, bilayers from thylakoid lipids remain remarkably good barriers against ions. The ion permeability of those lipids cannot explain the passive ion flux through thylakoid membranes. The thylakoid lipids are about as permeable as pure phospholipids to H^+: at least five orders of magnitude higher than alkali or halide ion permeability. Permeability coefficients for proton measured with galactolipids are close to that of the thylakoid membrane from which it is purified. They exhibit proton selectivity in HCl-generated pH gradients, indicating that H^+ was not transported as neutral HCl molecules.

B. Fuks is a Research Assistant, and F. Homblé is a Research Associate of the National Fund for Scientific Research (Belgium), which is kindly acknowledged for its financial support. The authors are greatly indebted to Prof. J. M. Ruysschaert for fruitful discussions.

REFERENCES

Andreoli, T. E., J. A. Bangham, and D. C. Tosteson. 1967. The formation and properties of thin lipid membranes from HK and LK sheep red cell lipids. *J. Gen. Physiol.* 50:1729–1749.

Andreoli, T. E., M. Tieffenberg, and D. C. Tosteson. 1967. The effect of valinomycin on the ionic permeability of thin lipid membranes. *J. Gen. Physiol.* 50:2527–2545.

Barber, J. 1972. Stimulation of millisecond delayed light emission by KCl and NaCl gradients as a means of investigating the ionic permeability properties of the thylakoid membranes. *Biochim. Biophys. Acta.* 275: 105–116.

Benz, R., O. Fröhlich, P. Laüger, and M. Montal. 1975. Electrical capacity of black lipid films and of lipid bilayers made from monolayers. *Biochim. Biophys. Acta.* 394:323–334.

Deamer, D. W., and W. J. Nichols. 1983. Proton-hydroxide permeability of liposomes. *Proc. Natl. Acad. Sci. USA.* 80:165–168.

Deamer, D. W. 1987. Proton permeation of lipid bilayers. *J. Bioenerg. Biomembr.* 19:457–479.

Dorne, A. J., J. Joyard, and R. Douce. 1990. Do thylakoids really contain phosphatidylcholine? *Proc. Natl. Acad. Sci. USA.* 87:71–74.

Enz, C., T. Steinkamp, and R. Wagner. 1993. Ion channels in the thylakoid membrane (a patch-clamp study). *Biochim. Biophys. Acta.* 1143:67–76.

Farkas, D. L., R. Korenstein, and S. Malkin. 1984. Electrophotoluminescence and the electrical properties of the photosynthetic membrane. *Biophys. J.* 45:363–373.

Flewelling, R. F., and W. L. Hubbel. 1986. The membrane dipole potential in a total membrane potential model. Applications to hydrophobic ions interactions with membranes. *Biophys. J.* 49:541–552.

Foley, A. A., A. P. R. Brain, P. J. Quinn, and J. L. Harwood. 1988. Permeability of liposomes composed of binary mixtures of monogalactosyldiacylglycerol and digalactosyldiacylglycerol. *Biochim. Biophys. Acta.* 939:430–440.

Gounaris, K., A. Sen, A. P. R. Brain, P. J. Quinn, and W. P. Williams. 1983. The formation of non-bilayer structures in total polar lipid extracts of chloroplast membranes. *Biochim. Biophys. Acta.* 728:129–139.

Graziani, Y., and A. Livne. 1972. Water permeability of bilayer lipid membranes: sterol-lipid interaction. *J. Membr. Biol.* 7:275–284.

Grzesiek, S., and N. A. Dencher. 1986. Dependency of pH-relaxation across vesicular membranes on the buffering power of bulk solutions and lipids. *Biophys. J.* 50:265–276.

Gutknecht, J., and D. C. Tosteson. 1970. Ionic permeability of thin lipid membranes. Effects of n-alkyl alcohols, polyvalents cations, and a secondary amine. *J. Gen. Physiol.* 55:359–374.

Gutknecht, J., and A. Walter. 1981a. Transport of protons and hydrochloric acid through lipid bilayer membranes. *Biochim. Biophys. Acta.* 641: 183–188.

Gutknecht, J., and A. Walter. 1981b. Hydroxyl ion permeability of lipid bilayer membranes. *Biochim. Biophys. Acta.* 645:161–162.

Gutknecht, J. 1984. Proton/hydroxide conductance through lipid bilayer membranes. *J. Membr. Biol.* 82:105–112.

Gutknecht, J. 1987. Proton conductance through phospholipid bilayers: water wires or weak acids? *J. Bioenerg. Biomembr.* 19:427–442.

Hall, J., C. A. Mead, and G. Szabo. 1973. A barriermodel for current flow in lipid bilayer membrane. *J. Membr. Biol.* 11:75–97.

Hauser, H., D. Oldani, and M. C. Phillips. 1973. Mechanisms of ion escape from phosphatidylcholine and phosphatidylserine single bilayer vesicles. *Biochemistry.* 12:4507–4517.

Hind, G., H. Y. Nakatani, and S. Izawa. 1974. Light-dependent redistribution of ions in suspensions of chloroplast thylakoid membranes. *Proc. Natl. Acad. Sci. USA.* 71:1484–1488.

Hopfer, U., A. L. Lehninger, and W. J. Lennarz. 1970. The effect of polar lipid moiety of lipids on the ion permeability of bilayer membranes. *J. Membr. Biol.* 2:41–58.

Junge, W. 1977. Membrane Potentials in photosynthesis. *Annu. Rev. Plant Physiol.* 28:503–536.

Junge, W., and J. B. Jackson. 1982. The development of electrochemical potential gradients across photosynthetic membranes. *In* Energy Conversion By Plants and Bacteria. Govindjee, editor. Academic Press, New York. 589–646.

Krishnamoorthy, G., and P. C. Hinkle. 1984. Non-ohmic proton conductance of mitochondria and liposomes. *Biochemistry.* 23:1640–1645.

Miyamato, V. K., and T. E. Thompson. 1967. Some electrical properties of lipid bilayer membranes. *J. Colloid Interface Sci.* 25:16–25.

Montal, M., and P. Mueller. 1972. Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. *Proc. Natl. Acad. Sci. USA.* 69:3561–3566.

Mueller, P., D. O. Rudin, H. Ti Tien, and W. C. Wescott. 1962. Reconstitution of cell membrane structure in vitro and its transformation into an excitable system. *Nature.* 194:979–980.

Nagle, J. F., and S. Tristram-Nagle. 1983. Hydrogen bonded chain mechanisms for proton conduction and proton pumping. *J. Membr. Biol.* 74:1–14.

Nagle, J. F. 1987. Theory of passive proton conductance in lipid bilayers. *J. Bioenerg. Biomembr.* 19:413–426.

Nichols, W. J., and D. W. Deamer. 1980. Net proton-hydroxyl permeability of large unilamellar liposomes measured by an acid-base titration technique. *Proc. Natl. Acad. Sci. USA.* 77:2038–2042.

Ohki, S. 1969. The electrical capacitance of phospholipid membranes. *Biophys. J.* 9:1195–1205.

Ohki, S. 1969. The variation of the direct current resistance of lipid bilayers. *J. Colloid Interface Sci.* 30:413–420.

Oldani, D., H. Hauser, B. W. Nichols, and M. C. Phillips. 1975. Monolayer characteristics of some glycolipids at the air-water interface. *Biochim. Biophys. Acta.* 382:1–9.

Pagano, R., and T. E. Thompson. 1968. Spherical lipid bilayer membranes: electrical and isotopic studies of ion permeability. *J. Mol. Biol.* 38:41

Pagano, R., J. M. Ruysschaert, and I. R. Miller. 1972. The molecular composition of some lipid bilayer membranes in aqueous solutions. *J. Membr. Biol.* 10:11

Portis, A. R. 1981. Evidence of a low stromal Mg^{2+} concentration in intact chloroplasts in dark. *Plant Physiol.* 67:985–989.

Robertson, R. N., and T. E. Thompson. 1977. The function of phospholipid polar groups in membranes. *FEBS Lett.* 76:16–19.

Redwood, W. R., F. R. Pfeiffer, J. A. Weisbach, and T. E. Thompson. 1971. Physical properties of bilayer membranes formed from a synthetic saturated phospholipid in n-decane. *Biochim. Biophys. Acta.* 233:1–6.

Reyes, J., and R. Latorre. 1979. Effects of the anesthetics benzyl alcohol and chloroform on bilayers made from monolayers. *Biophys J.* 28:259–280.

Rossignol, M., P. Thomas, and C. Grignon. 1982. Proton permeability of liposomes from natural phospholipid mixtures. *Biochim. Biophys. Acta.* 684:195–199.

Schönfeld, M., and H. Schickler. 1984. The permeability of the thylakoid membrane for protons. *FEBS Lett.* 167:231–234.

Schönknecht, G., R. Hedrich, W. Junge, and K. Raschke. 1988. A voltage-dependent chloride channel in the photosynthetic membrane of a higher plant. *Nature.* 336:589–592.

Shipley, G. G., J. P. Green, and B. W. Nichols. 1973. *Biochim. Biophys. Acta.* 311:531–544.

Simon, S. A., L. J. Lis, R. C. McDonald, and J. W. Kauffman. 1977. The non effect of large hydrocarbon, squalene, on the phosphatidylcholine packing structure. *Biophys. J.* 19:83–90.

Sprague, S. G., and L. A. Staehelin. 1984. Effects of reconstitution method on structural organization of isolated chloroplast membrane lipids. *Biochim. Biophys. Acta.* 777:306–322.

Tester, M., and M. R. Blatt. 1989. Direct measurements of K^+ channels in thylakoid membranes by incorporation of vesicles into planar lipid bilayers. *Plant. Physiol.* 91:249–252.

Tien, T. H. 1974. Bilayer Lipid Membranes (BLM). Theory and Practice. Marcel Dekker, Inc., New York. 655 pp.

Toyoshima, Y., and T. E. Thompson. 1975. Chloride flux in bilayer membranes: the electrically silent chloride flux in semispherical bilayers. *Biochemistry.* 14:1518–1524.

Van Kooten, O., J. F. Snel, and W. J. Vredenberg. 1986. Photosynthetic free energy transduction related to the electric potential changes across the thylakoid membrane. *Photosynthesis Res.* 9:211–227.

Vredenberg, W. J. 1976. Electrical interactions and gradients between chloroplast compartments and cytoplasm. *In* The Intact Chloroplast. J. Barber, editor. Elsevier, North-Holland. 53–88.

Webb, M. S., and B. R. Green. 1989. Permeability properties of large unilamellar vesicles of thylakoid lipids. *Biochim. Biophys. Acta.* 984:41–49.

Webb, M. S., and B. R. Green. 1991. Biochemical and biophysical properties of thylakoid acyl lipids. *Biochim. Biophys. Acta.* 1060:133–158.

White, S. H., D. C. Petersen, S. Simon, and M. Yafuso. 1976. Formation of planar bilayer membranes from monolayers. A critique. *Biophys. J.* 16:481–489.