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THE INFLUENCE OF PHOSPHOLIPID POLAR GROUPS ON GRAMICIDIN CHANNELS

ERWIN NEHER * AND HANSJÖRG EIBL

Max-Planck-Institut für Biophysikalische Chemie, (Karl-Friedrich-Bonhoeffer-Institut) D 34 Göttingen (G.F.R.)

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Summary

The influence of well-defined changes in the polar part of phospholipid molecules on the properties of black lipid membranes was studied using a series of phospholipids with identical hydrocarbon chains, but systematically changed polar groups. The hydrocarbon tails of the lipids under study were composed of 1,2-dipentadecylmethylidene glycerol. The polar parts differed in the degree of *N*-methylation and comprised phosphocholine, -*N*,*N*-dimethylethanolamine, -*N*-methylethanolamine and ethanolamine. Stable black lipid membranes could be formed with the solvents octane, decane, dodecane, tetradecane and hexadecane. The properties of gramicidin-induced single ionic channels changed systematically in membranes from the phosphatidylcholine to the phosphatidylethanolamine analogue, as indicated by an increase in the amplitude Λ of the unit conductance step and a decrease in the average channel life-time or duration τ. The series of τ-values was opposite to that expected from hydrocarbon thickness (specific capacitance). It is suggested that the surface tension γ is a relevant parameter for the prediction of τ-values.

Introduction

The recording of discrete conductance steps due to opening and closing of individual gramicidin A channels in black lipid membranes has been reported for a number of different lipids [1—4]. The amplitude Λ of the unit conductance step was found to depend quite strongly on the lipid used [2—4]. The mean lifetime of an open channel τ, however, was influenced mainly by the hydrocarbon in which the phospholipid had been dissolved for membrane formation [1]. This was interpreted as the influence of membrane thickness, since

* To whom correspondence should be addressed at: Max-Planck-Institut für biophysikalische Chemie (Karl-Friedrich-Bonhoeffer-Institut) Am Fassberg, D 34 Göttingen-Nikolausberg (G.F.R.)

more or less solvent is incorporated into a black lipid membrane [5] depending on the chain-length of the solvent.

In this investigation the quantities Λ and τ were measured for membranes made from different phospholipids, all of which were identical in their hydrocarbon tails (see Materials and Methods). The polar headgroups of the phospholipids, however, were changed systematically by reducing stepwise the degree of *N*-methylation from phosphatidylcholine (phosphatidyl-*N*,*N*,*N*-trimethylethanolamine) to phosphatidylethanolamine. This was considered to be an interesting change in the molecular structure of phospholipids, complementary to investigations where hydrocarbon tails were varied systematically leaving the ionic headgroups unchanged [6,7].

Materials and Methods

The synthesis of the phospholipids used in this study is described elsewhere [8]. A general intermediate for the synthesis of all of the four phospholipids, the β-bromoethylester of 1,2-dipentadecylmethylidene glycero-3-phosphoric acid, was used, and the phospholipids were obtained by one amination step with the amines trimethylamine, dimethylamine, methylamine and ammonia, to ensure that the apolar part of the four molecules had an identical composition (Fig. 1). 90°-light scattering was used to indicate the lipid phase transition [9]. Values for T_t of 18, 22, 32, and 35° C were found for phosphatidylcholine, *N*,*N*-dimethylcephalin, *N*-methylcephalin and phosphatidylethanolamine, respectively. Other materials used were the same as described earlier [4].

Black lipid membranes can be formed from these lipids using the solvents octane, decane, dodecane, tetradecane and hexadecane. Membranes made from hexadecane and the phosphatidylethanolamine analogue or the *N*-methylethanolamine analogue are quite unstable, however. The membranes were formed from 2 to 5 mM solutions after pre-painting the membrane support with 10 mM solutions of the lipid in ethanol. Gentle heating of the solutions prior to membrane formation is required for some solvent-lipid combinations in order to obtain clear solutions in the concentration range stated.

All experiments were performed in 1 molar KCl with an experimental arrangement as described in a previous communication [4]. Current at a constant membrane voltage of 100 mV was recorded by a pen recorder or alternatively on a storage oscilloscope. Single step conductance values were obtained by dividing the most frequently occurring unit current step by the applied voltage (0.1 V). Mean lifetimes (or durations) of the channels were determined by calculating the autocorrelation function of current fluctuations by means of a

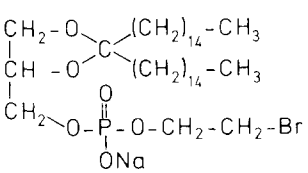

Fig. 1. A general intermediate, the β-bromoethylester of 1,2-dipentadecylmethylidene glycero-3-phosphate, for the synthesis of phospholipid analogues by direct amination.

Saicor SAI-42a autocross-correlator [4]. This was done at very low mean current levels, at which discrete conductance steps could clearly be seen by visual observation. The time constant of the autocorrelation function was taken as the mean channel lifetime τ. In many cases the validity of this procedure was checked by carrying out both the correlation-measurement and manual determinations of lifetimes of 50 to 100 individual channels from oscilloscope traces. Values from both methods agreed within 20%.

For some solvent-lipid combinations (for instance octane and the analogue of *N*,*N*-dimethylethanolamine; decane and the analogue of *N*-methylethanolamine) channel lifetimes were quite variable in so far as they depended on the age of the membrane and the degree of pre-painting. Changes were as much as approximately 50%. In these cases average values from several determinations between 5 and 40 min after membrane formation are given.

Specific capacitance was measured with membranes of approx. 1 mm diameter. Membrane diameter, or, in case of ovally shaped membranes, half axes of the ellipse, were determined by a calibrated eyepiece graticule of a Leitz stereo microscope. One side of this microscope was used as an illumination pathway, the other one for viewing the membrane under reflected light. Capacitance was measured with a Wayne-Kerr impedance bridge at 2 kHz; 40 mV_{rms}.

Membrane tension γ was determined with the same optical arrangement as used for capacitance measurements. A second light source was added as illustrated in Fig. 2 and after forming the membrane and inflating it by hydrostatic pressure, the lateral distance of the two faint reflections originating from the light sources was determined. Optimal accuracy was obtained when the microscope was focused such that sharp images of the two light sources were obtained. In this case the inflated membrane can be considered as a concave mirror, and a simple relation between the radius of curvature r, the lateral distance between the two images a, and the angle α between both illuminating light beams is obtained:

$$r = \frac{a}{2 \sin \alpha/4} \tag{1}$$

This relation assumes the light sources to be far away from the mirror (see legend Fig. 2). Hydrostatic pressure was increased by withdrawing electrolyte from the outer compartment with a microliter syringe, until a steady state with a radius of curvature 1.55 mm was obtained as determined through Eqn. 1. The volume change with repect to a flat membrane was read from the microliter syringe and converted to a change in pressure. The reference state of a flat membrane was determined by a uniform faint reflectance across the whole membrane. Membrane tension γ was calculated according to the Laplace-Young equation:

$$\gamma = r \cdot \Delta p/2 \tag{2}$$

where Δp is the hydrostatic pressure difference. It should be noted that membranes were in the black state during these measurements. Only the very faint residual reflectance was used. For better visibility, chambers milled from black "Dynal" were used for membrane tension measurements. Prerequisite to the method were very carefully drilled and polished holes with uniform edges, and

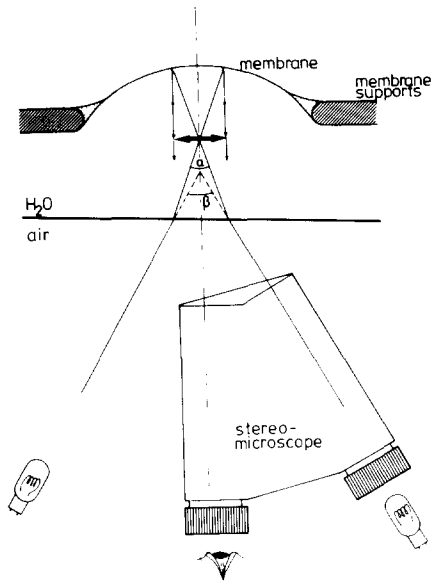

Fig. 2. Experimental setup for the determination of membrane tension. One optical pathway of a Leitz stereo microscope was used for illumination. An additional light source was mounted to the stereo microscope in the plane of the two optical pathways, such that the two central beams of illumination included an angle of 30° in air. From that an angle of 22° between both beams in 1 M KCl was calculated. When an inflated membrane was viewed with this arrangement, images of the two light sources could be seen, if the microscope was focused on a plane that contained the focal point of the hollow mirror (the inflated membrane!). The lateral distance between the centres of both images is connected to the radius of curvature of membrane through a simple relationship, as given in the text. This relationship should be corrected for small asymmetries between the two illumination beams with respect to the viewing beam (the wall of the cuvette is not orthogonal to the viewing beam in order to eliminate reflections from the glass wall). However, the corrections are irrelevant, since only small angles (5—10°) are involved.

toruses free of contaminating particles. Irregularities therein showed up as distortions in the image formation. Membranes that gave unsharp and unfaithful images of the light sources (end faces of cylindrical bundles of glass fibers) were rejected.

The method described here has the advantage that the membrane has to be inflated only minimally. It turned out that steady-state conditions for reproducible measurements could be obtained more easily than with the maximum bubble-pressure method [10]. For a given membrane in a steady state, and tensions above 3 dyne/cm, determination of γ was reproducible within ±5%.

Results and Discussion

Very stable black lipid membranes can be formed at temperatures near room temperature (20—41°C). Liquid crystalline phase transitions of the pure lipids occur between 18 and 35°C (see Materials and Methods). Measurements were performed at 34°C except where stated otherwise, and the membranes were expected to be in the fluid state, since it can be assumed that transition temperatures are lowered by the presence of solvent. No discontinuities of membrane parameters could be observed when approaching a temperature range in which a phase change was expected to occur, although the membranes became

very fragile. Average lifetimes of the channels in membranes made from the phosphatidylethanolamine-analogue were measured as a function of temperature in the range of 24 to 41° C. When log τ is plotted versus $1/T$, the data can be well approximated by a straight line, the slope of which suggests an activation energy of 19.5 kcal/mol. This value is very similar to values reported for membranes of dioleoyl phosphatidylcholine and 1-oleoylglycerol ($\leqslant$ 19 kcal/mol [3]). Likewise the temperature dependence of the single channel conductance Λ is described by an activation energy of 6.9 kcal/mol, a value very similar to that in dioleoyl phosphatidylcholine.

The discrete conductance steps produced by gramicidin A are similar to those observed in membranes made from other phospholipids. The size of the steps, however, is larger. The value for the phosphatidylcholine analogue ($4.1 \cdot 10^{-11}$ siemens) exceeds values reported for dioleoylphosphatidylcholine ($3 \cdot 10^{-11}$ siemens, calculated from [4] for a temperature of 34° C). Channel size increases systematically from 1,2-dipentadecylmethylidene glycero-3-phosphocholine to -ethanolamine (Fig. 3). Likewise, the average channel lifetime changes systematically with the lipid (Fig. 3). For example, with decane as the solvent, the values for the phosphatidylcholine and phosphatidylethanolamine analogues are 1.4 ± 0.25 s (mean ± S.E.) and 0.17 ± 0.02 s, respectively.

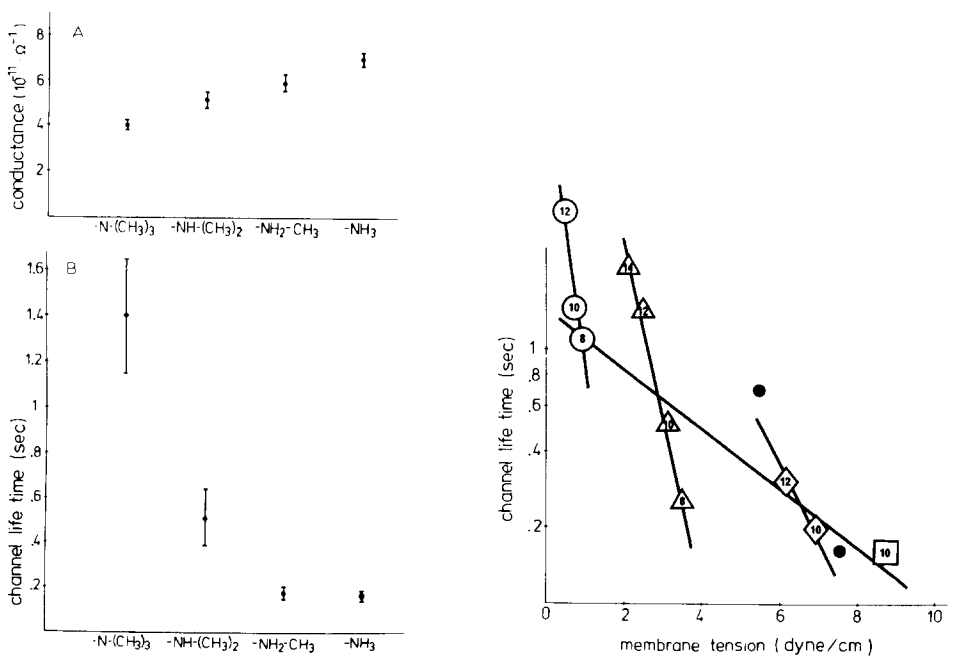

Fig. 3. Amplitude of the unit conductance step (A) and average channel lifetime (B) for the different lipids dissolved in decane at 34° C, 1 M KCl. Error bars represent S.E.

Fig. 4. Average channel lifetime is plotted against membrane tension for membranes made from various combinations of lipid and solvent. The symbols represent lipids with dipenradecylmethylidene glycerol tails and different polar groups:-○-, phosphocholine; -Δ-, *N,N*-dimethylethanolamine; -◇-, *N*-methylethanolamine; -□-, ethanolamine; solvents are given by numbers within the symbols, an 8 indicating octane etc. Values for 1-oleoylglycerol membranes with decane and hexadecane as solvents are included in the figure for comparison, -●-.

Differences in channel lifetimes are reminiscent of similar differences that were observed when membranes of 1-oleoyl glycerol were made with different solvents [1]. These changes were explained by the assumption that channel opening times are very sensitive indicators of membrane thickness, since different solvents lead to membranes of different thickness (different solvents are incorporated to varying degrees into the membranes). Channel lifetimes have been reported to be shorter for thicker membranes.

Making the additional assumption that the above relationship is also true for different lipids with small and systematic variations in the polar part of the molecule, we tried to correlate our results on channel lifetimes with the values for specific capacitance. It turned out that the series of C-values varied in a fashion opposite to that expected from determinations of channel lifetimes (see Table I). On the other hand, a series of determinations of both channel lifetimes and specific capacitances with the same lipid but different solvents gave results as expected from earlier investigations on membranes of 1-oleoylglycerol (see Table I).

During the course of these experiments it was noticed that in all cases the channel lifetime seemed to be correlated with the membrane tension, with a shorter channel duration as the tension increased. This seemed to be reasonable, since surface tension strongly influences the chemical potential of surface active substances [12]. This implies that equilibrium and rate constants of reactions at the interface are proportional to $\exp(\gamma \Delta a/RT)$, where Δa is the difference between the partial molar surface areas of product and reactants. A linear relationship between log τ and γ would be expected if γ is the only factor influencing channel lifetime. We therefore measured membrane tension (see Materials and Methods) for different lipid-solvent combinations. The results substantiated

TABLE I

SPECIFIC CAPACITANCE AND MEAN CHANNEL LIFETIME τ FOR DIFFERENT COMBINATIONS OF LIPID AND SOLVENT AT 34°C

Mean ± S.E. (number of measurements)

Different lipids with decane as solvent

Lipid	Phosphatidyl-choline	*N,N*-dimethyl-phosphatidyl-ethanolamine	*N*-methyl-phosphatidyl-ethanolamine	Phosphatidyl-ethanolamine	1-oleoyl glycerol
Spec. capacity ($\mu F/cm^2$)	0.386 ± 0.006 (6)	0.445 ± 0.003 (11)	0.49 ± 0.01 (6)	0.493 ± 0.008 (14)	0.424 ± 0.008 (9) 0.419 *
τ (s)	1.4 ± 0.25 (9)	0.5 ± 0.1 (9)	0.15 ± 0.02 (5)	0.17 ± 0.02 (5)	—

N,N-dimethylethanolamine-analogue and different solvents

Solvent	Octane	Decane	Dodecane
Spec. capacity ($\mu F/cm^2$)	0.447 ± 0.004 (6)	0.45 ± 0.01 (6)	0.578 ± 0.004 (8)
τ (s)	0.27 ± 0.03 (10)	0.51 ± 0.08 (9)	1.4 ± 0.2 (6)

* From Andrews et al. ref. 11, measured in saturated NaCl at room temperature.

our hypothesis, as is shown in Fig. 4, where the logarithm of τ is plotted versus membrane tension. When considering the values for membranes from decane solutions of the different lipids, a systematic relation is obvious. Moreover, the same trend appears when considering membranes made from the same lipid and different solvents; however the relation between τ and γ is steeper. Included in Fig. 4 are values for 1-oleoylglycerol, which fit into the general scheme.

Several properties make the series of lipids described here an interesting tool in membrane research. First of all they are identical in their hydrocarbon tails and vary systematically in their polar part. This should be helpful in studying the influence of polar groups on membrane properties. Secondly, the hydrocarbon tails are devoid of double bonds, which confers chemical stability to the lipids. Thirdly, the lipids show crystalline liquid phase transitions in a favourable temperature range (18—35° C) which makes it possible to work well above and near the transition.

The most surprising finding was that the lifetime of gramicidin channels increased with increasing hydrocarbon thickness, in contrast to what is expected if results from mono- and diglycerids are generalized. On the other hand, membrane tension was found to be strongly correlated with the channel lifetime. For a fixed lipid, varying solvents, this influence is intensified by the concomitant change in membrane thickness, whereas the influences of membrane thickness and surface tension counteract each other if membranes are prepared from decane solutions of different lipids.

Considering the influence of membrane tension on the lifetime of gramicidin channels also resolves a discrepancy between theoretical expectations and experiments. The hypothesis that channel lifetime τ is a function of membrane thickness only predicts an approx. 50% increase in τ when changing the voltage by 100 mV. This rough estimate is obtained by taking the relation between τ and membrane thickness from Hladky and Haydon [1] and inserting into it the change in thickness brought about by 100 mV applied potential as obtained by capacitance measurements [11]. Contrary to this expectation no voltage-dependence of τ has been found in the range 0 to 100 mV [1,13]. On the other hand, changes in voltage ΔV are connected with changes in membrane tension $\Delta\gamma$ via the Lippman equation [14].

$$\Delta\gamma = 0.5 \cdot C_{spec} \cdot \Delta V^2 \qquad (3)$$

With an average specific capacitance of 0.4 $\mu F/cm^2$ and an applied potential of 200 mV, (3) predicts a change in membrane tension of 0.08 dyne/cm. According to Fig. 4 this small change leads only to a negligible change in τ, which is in agreement with experimental observation.

References

1 Hladky, S.B. and Haydon, D.A. (1972) Biochim. Biophys. Acta 274, 294—312
2 Goodall, M.C. (1973) Arch. Biochem. Biophys. 157, 514—519
3 Bamberg, E. and Läuger, P. (1974) Biochim. Biophys. Acta 367, 127—133
4 Zingsheim, H.P. and Neher, E. (1974) Biophys. Chem. 2, 197—207
5 Fettiplace, R., Andrews, D.M. and Haydon, D.A. (1971) J. Membrane Biol. 5, 277—296
6 Benz, R., Stark, G., Janko, K. and Läuger, P. (1973) J. Membrane Biol. 14, 339—364
7 Bamberg, E. and Benz, R. (1976) Biochim. Biophys. Acta 426, 570—580

8 Eibl, H. and Nicksch, A. (1976) Chem. Phys. Lipids, in the press
9 Träuble, H. (1972) in Biomembranes (Kreutzer, O.O. and Siegers, J.F.G., eds.), Vol. 3, pp. 197—227, Plenum Publishing Comp., New York
10 Tien, H.T. (1967) J. Physical. Chem. 71, 3395—3412
11 Andrews, D.M., Manev, E.D. and Haydon, D.A. (1970) Special Disc. Farad. Soc. 1, 46—54
12 Aveyard, R. and Haydon, D.A. (1973) An Introduction to the Principles of Surface Chemistry, University Press, Cambridge
13 Bamberg, E. and Läuger, P. (1973) J. Membrane Biol. 11, 177—194
14 Requena, J., Haydon, D.A. and Hladky, S.B. (1975) Biophys. J. 15, 77—81