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# The Effect of the Polar Moiety of Lipids on Bilayer Conductance Induced by Uncouplers of Oxidative Phosphorylation

ULRICH HOPFER, ALBERT L. LEHNINGER, and W. J. LENNARZ

Department of Physiological Chemistry,
The Johns Hopkins University School of Medicine,
Baltimore, Maryland 21205

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*Summary.* Bilayer membranes were formed from decane, cholesterol, and three lipids isolated from *Staphylococcus aureus:* positively charged lysyl phosphatidylglycerol (LysPG), negatively charged phosphatidylglycerol (PG), and neutral diglucosyldiglyceride (DiGluDiGly). The uncouplers of oxidative phosphorylation, 2,4-dinitrophenol (DNP) and 3-t-butyl,5-chloro,2′-chloro,4′-nitrosalicylanilide (S 13), increased the electrical conductance of all three differently charged bilayers. S 13 was found to be the most effective reagent of the known uncouplers in increasing conductance of the bilayers. The conductance induced by uncouplers was investigated as a function of pH and uncoupler concentration. The pH of maximum conductance for each uncoupling agent was dependent on both the uncoupler and the lipid; it was lower for each uncoupler in LysPG and higher in PG compared to DiGluDiGly bilayers. At a pH below the optimum for LysPG, the conductance of the positively charged membrane was 500 times and of the neutral one 10 times higher than that of the negatively charged bilayer at equal uncoupler concentration and pH. Above the pH optimum for DiGluDiGly, the conductance was approximately equal for the positive and neutral membranes, but was lower in PG bilayers. Conductance depended linearly on uncoupler concentration. The bilayer conductance induced by S 13 was entirely due to increased proton permeability in all three lipids. The findings are consistent with the role of uncouplers as "carriers" for protons across the hydrocarbon interior of lipid membranes. The differences in conductance of differently charged lipid bilayers at equal uncoupler concentration, as well as the change of pH optimum of conductance with lipid charge, can be explained in terms of an electrostatic energy contribution of the fixed lipid charges to the distribution of the uncoupler anion between the aqueous and the membrane phases.

Lipid bilayers have been used as models to investigate the ways in which small lipophilic molecules can alter the properties of natural membranes (*see* reviews of Bangham, 1968, and of Henn & Thompson, 1969). Noteworthy among the classes of molecules that affect membranes are the un-

couplers of oxidative phosphorylation (Parker, 1965). Three classes of uncouplers – substituted phenols, benzimidazoles, and carbonylcyanide phenylhydrazones – have been tested on synthetic lipid membranes (Hopfer, Lehninger & Thompson, 1968; Liberman & Topaly, 1968; Lea & Croghan, 1969), and all three types have been shown to increase the electrical conductance of bilayers.

All of the known uncouplers are weak organic acids containing $\pi$-orbitals, which can serve to delocalize the charge of the ionized form (Mitchell, 1968). Such delocalization of charge energetically favors the solubility of an ion in a medium of low dielectric (Parsegian, 1969). It has been observed that the increase in conductance produced by uncouplers is pH dependent; it is optimum at or above the pK of the acid. In the region of the optimum, the uncoupler-induced conductance is almost entirely due to a high proton permeability as measured by a high proton diffusion potential (Hopfer *et al.*, 1968). Based on these and other observations, it has been suggested that the uncoupler anion, or a negatively charged complex containing the anion, increases conductance of bilayer membranes by carrying the electrical current within the membrane (Markin, Kristalik, Liberman & Topaly, 1969; Lea & Croghan, 1969).

Recently we have reported that the polar head group of the phospholipids plays an important role in determining the permselectivity of lipid bilayers to ions; bilayers of positively charged lipids are anion selective, whereas bilayers of negatively charged lipids are cation selective (Hopfer, Lehninger & Lennarz, 1970). In view of these findings, it would be expected that the fixed lipid charge might be a determining factor in the efficacy of the uncoupler in increasing membrane conductance if the concentration of the anionic form of the uncoupler within the membrane is the limiting molecular species. In these studies the effect of two uncouplers [2,4-dinitrophenol (DNP) and 3-t-butyl,5-chloro,2′-chloro,4′-nitrosalicylanilide (S 13) (Williamson & Metcalf, 1967)] on bilayers prepared with three differently charged lipids, all isolated from *Staphylococcus aureus*, have been studied. The lipids utilized were: diglucosyldiglyceride (DiGluDiGly), a neutral glycolipid; phosphatidylglycerol (PG), a negatively charged phospholipid; and lysyl phosphatidylglycerol (LysPG), a positively charged phospholipid.

An attempt to determine the influence of fixed charge on the efficacy of the uncouplers was of special interest since bacteria, which contain a higher ratio of negatively charged to neutral lipids than mitochondria, are known to be relatively insensitive to uncouplers of mitochondrial phosphorylation (Kates, 1964; Gel'man, Lukoyanova & Ostrovskii, 1967).

## Materials and Methods

Distilled water was passed over a mixed-bed ion-exchange resin and redistilled. Analytical reagent grade KCl was recrystallized from a solution of $10^{-3}$ M ethylenediaminetetraacetate (EDTA). Other chemicals were of highest purity available. S 13 was a gift of Monsanto Chemical Co. (St. Louis, Mo.). DNP was purchased from Eastman Kodak Co. (Rochester, N.Y.). Stock solutions of S 13 ($10^{-2}$ M) and of DNP (0.185 M) were prepared in absolute ethanol.

The purification of lipids and the techniques for membrane formation and electrical measurement have been previously described (Hopfer *et al.*, 1970). Electrical conductance was determined from the current caused by an impressed voltage of $\pm 20$ mV. Corrections were made, if necessary, for electrode resistance (approximately 200 kΩ). The membrane-forming solution consisted of a dispersion of 1% purified lipid and 0.4% cholesterol in decane. The bilayer was formed over a hole of 2-mm diameter in a Plexiglas septum separating two aqueous compartments.

Two different kinds of cells were employed: one cell consisted of two chambers, each containing 10 ml of fluid, open to the atmosphere (Thompson, 1964); the other cell contained one chamber of 15 ml open to the atmosphere and a second of 2-ml volume that was closed (Hopfer *et al.*, 1970). The first design was used if additions had to be made to both chambers during an experiment after formation of the bilayer. The latter was used in all other instances. The chambers were stirred magnetically, and maintained at $25 \pm 1°$ C. The chambers contained $1.0 \times 10^{-2}$ M KCl and $10^{-5}$ M EDTA buffered with $10^{-3}$ M $KH_2PO_4$, $10^{-3}$ M citric acid, or $10^{-3}$ M Tris-HCl. The pH was adjusted with 1 N HCl or 1 N KOH to the desired value. The ionic strength varied between 0.011 and 0.017 M depending on the ionization of the buffer. The uncouplers were usually added to the buffer only after formation of the bilayer. However, the uncouplers were present in the buffer before membrane formation in the experiments in which the cell with one closed chamber was used. These experiments included measurements of proton diffusion potentials and of conductance involving no pH changes after bilayer formation. The chamber concentrations of uncoupler were calculated from the amount added and known solution volumes, and it was assumed that the amount partitioned into the lipid phase was negligible because of its small volume. The final ethanol concentration in the buffer did not exceed 0.05% (v/v).

In order to study membrane properties as a function of aqueous proton concentrations, changes of the buffer pH were carried out in two ways: (1) small equal amounts of 1 N HCl or KOH were added to both aqueous compartments after bilayer formation, and the new pH value was determined by a pH electrode in one of the chambers; or (2) a number of bilayers were successively made in a buffer whose pH was changed stepwise before formation of each membrane.

The dissociation constant of S 13 was determined in dimethylsulfoxide-water 20:80 (v/v) by measuring the difference in absorbancy at 376 nm between a solution of S 13 at pH 2 and a series of solutions of different but known pH values. The dissociation constant was calculated from the apparent $pK_a$ in dimethylsulfoxide-water, correcting for the decreased concentration of water.

## Results

### *Properties of Lipid Bilayers in the Absence of Uncoupling Agents*

As was found earlier (Hopfer *et al.*, 1970), bilayer membranes formed from the three differently charged lipids – PG, LysPG, DiGluDiGly – have

similar low conductances of $10^{-8}$ to $10^{-9}$ mho $cm^{-2}$. No significant effect of pH on conductance could be detected when conductance was measured at the same pH at which the bilayer had been formed.

However, the charged membranes made from PG or LysPG became mechanically unstable and showed large increases of conductance (up to 100-fold) if buffer pH or ionic strength were changed after the formation of the bilayer in such a manner that electrostatic repulsion between the lipid molecules decreased: e.g., PG membranes made at pH 7 broke consistently when the pH was lowered to 4.8, presumably because some lipid molecules lost their normal negative charge. A conductance increase preceded the breakage. LysPG membranes exhibited a similar behavior when they were formed at pH 5 and the buffer pH was subsequently increased above 7. The increased conductance observed under these conditions was clearly an artifact due to manipulations which decreased the mechanical stability of bilayers.

On the other hand, pH changes which increased the repulsive forces among the charged lipid molecules had no effect on stability and conductance but decreased the visible bilayer area. For example, PG membranes formed at pH 2 were very stable and their conductance remained low when the pH was raised to 7; their DC-capacitance, however, dropped to half the original value.

### *Properties of Lipid Bilayers in the Presence of Uncouplers*

As had been shown previously for other classes of uncouplers of oxidative phosphorylation (Hopfer *et al.*, 1968; Liberman & Topaly, 1968), the salicylanilide S 13 was very effective in raising the conductance of lipid bilayer membranes. S 13 at a concentration of $5 \times 10^{-8}$ M increased the conductance by a factor of $5 \times 10^3$ in a neutral (glycolipid) membrane. Under the conditions of these experiments, the current-voltage relationship was found to be linear up to $\pm 40$ mV of the impressed potential (Fig. 1). The magnitude of the effect of the uncouplers was found to be dependent on buffer pH, ionic strength and on the lipid used for bilayer formation. When the bilayer conductance in the presence of uncouplers was measured as a function of pH, it was necessary to avoid the above-mentioned artifacts owing to membrane instability. Therefore, measurements were obtained on a series of bilayers formed at different pH values or, in the presence of preformed bilayers, the pH was varied in the direction so as to increase membrane stability. In Fig. 2, the strong pH dependence of conductance when S 13 was present is shown. The pH that afforded a maximum effect was between 8.3 and 8.9 for a neutral glycolipid membrane, between 5.7 and 6.4 for the

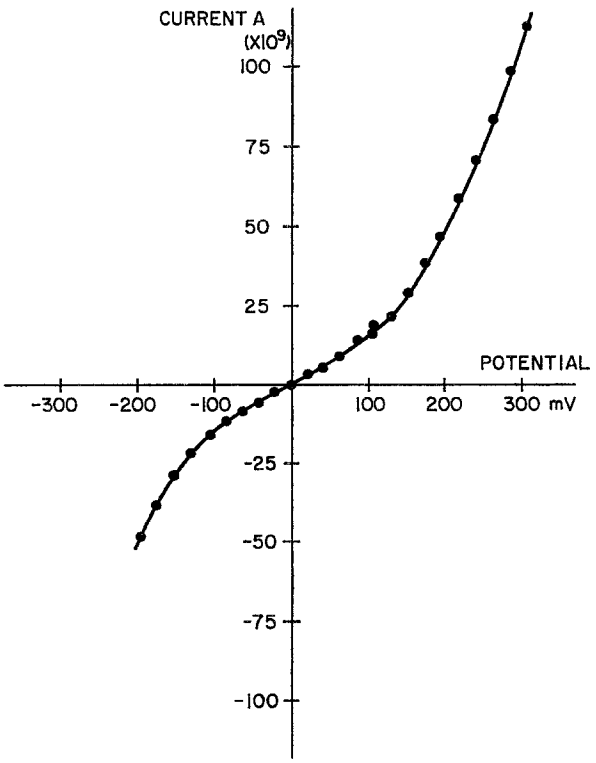


Fig. 1. Current-voltage relationship of a DiGluDiGly bilayer in presence of $1 \times 10^{-8}$ M S13

positively charged LysPG, and between 9.4 and 9.8 for the negatively charged PG. It is also evident from Fig. 2 that S 13 increased conductance at pH values below the optimum to a different extent in the three differently charged membranes. It should be noted that in the case of the LysPG membrane, the amount of salicylanilide employed was only 1/50th of that used for DiGluDiGly and PG membranes, because higher concentrations increased conductance so much that it could no longer be measured accurately with the available instruments.

To evaluate the effect of lipid charge more exactly, conductance was measured as a function of S 13 concentration at a fixed pH of 6.4 for all three lipids (Fig. 3). The negatively charged PG membrane was the least sensitive to uncoupler; S 13 caused a 10-fold greater increase in the conductance of the neutral glycolipid membrane and a 500-fold greater increase in the positively charged LysPG membrane than in the PG bilayer. From Fig. 2 it can be inferred that this is valid at all pH values below 6.4, the pH optimum of LysPG membranes, as long as the inherent conductance

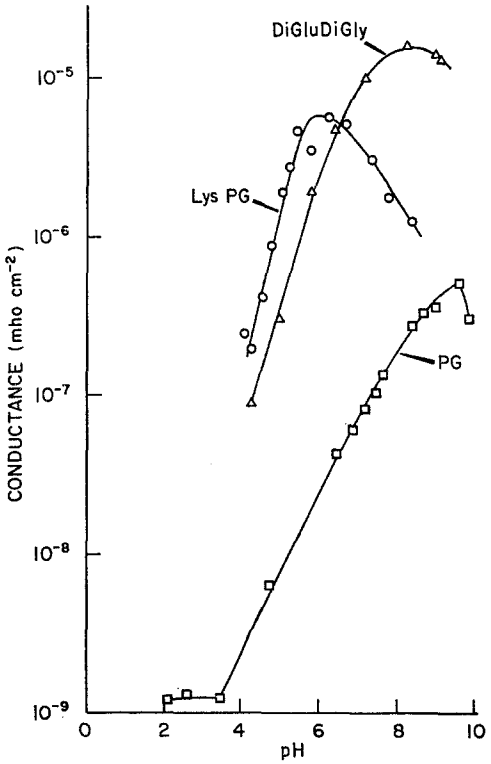


Fig. 2. pH dependence of bilayer conductance in presence of S 13. LysPG with $1 \times 10^{-9}$ M S 13 (o——o); DiGluDiGly with $5 \times 10^{-8}$ M S 13 (Δ——Δ); PG with $5 \times 10^{-8}$ M S13 (□——□

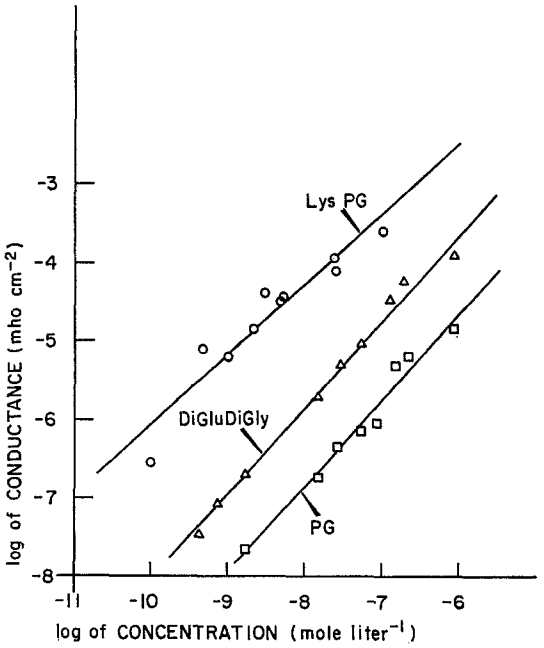


Fig. 3. Concentration dependence of conductance in presence of S 13 at pH 6.4 and ionic strength of 0.012 M

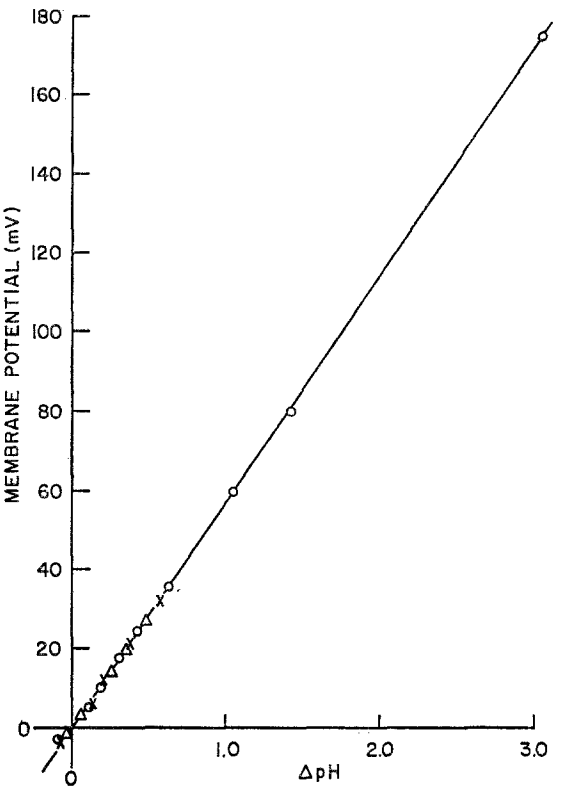


Fig. 4. Membrane potential as a function of pH gradient across the membrane in presence of S 13. The buffer was composed of 10 mM KCl, 10 μM EDTA, 1 mM Tris-HCl, 1 mM $KH_2PO_4$. The initial pH on both sides of the membrane was 6.7. DiGluDiGly (o——o); PG (×——×); LysPG (Δ——Δ)

is small compared to the one induced by uncouplers. As shown in Fig. 3, the conductance depended linearly on the concentration of S 13 over the range studied. A least squares analysis for the slope of the plot in Fig. 3 gave values of 1.1 for PG and DiGluDiGly and 0.9 for LysPG.

It had been shown that the increased conductance of bilayers caused by uncouplers such as DNP was due to an increased proton (or hydroxyl ion) permeability (Hopfer *et al.*, 1968). The high proton permeability had been calculated from the measured proton diffusion potential, measured under conditions of low proton concentration compared to salt and buffer. To investigate if lipid charge had an influence on the mechanism of conductance by uncouplers, the same technique was applied to membranes of the three different lipids in the presence of S 13. The membrane potential was determined as a function of a pH gradient across the membrane in the range of pH 6.7 to 9.7. The bilayer was first formed in the usual buffer

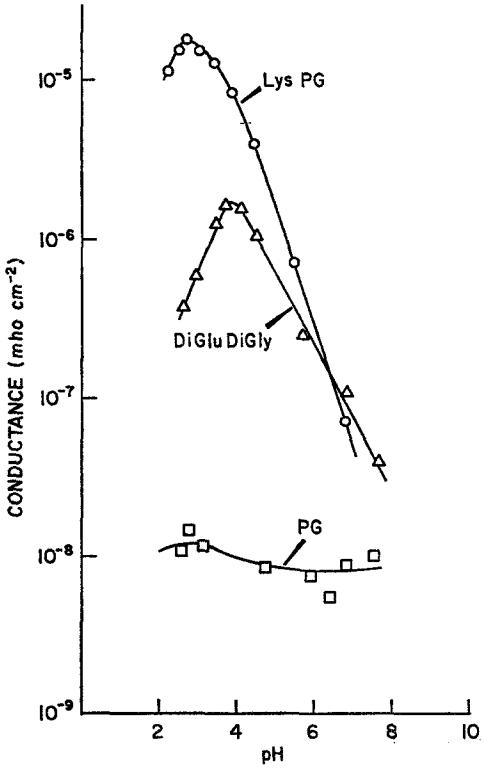


Fig. 5. pH dependence of bilayer conductance in presence of $9.3 \times 10^{-5}$ M DNP

containing 10 mM KCl, 10 μM EDTA, 1 mM Tris-HCl, and 1 mM phosphate buffer; then the gradient was established by changing the pH on only one side. The pH change was measured by a glass electrode. The magnitude and sign of the membrane potential in the presence of S 13 was the same for all three lipids (*see* Fig. 4). It had the sign of a proton potential and a value of 58.3 mV per pH unit, which is very close to the theoretical value of 59.6 mV per pH unit for membranes permeable to protons and to no other ions. This indicates that in the presence of S 13 all three kinds of bilayer membranes, regardless of the charge, become selectively permeable to protons.

It was of interest to determine if a lipid charge effect on conductance could also be observed in the presence of DNP; in addition, because of its low pK, DNP was better suited than the salicylanilide for investigating the effect in the pH range above the optimum. The pH optimum of conductance induced by DNP has been reported between 3.9 and 4.2 using phosphatidylcholine (Hopfer *et al.*, 1968) and brain lipid extract (Liberman & Topaly, 1968). In Fig. 5, the pH dependence of bilayer conductance in the

Table 1. *Effect of* $10^{-3}$ M *DNP on membrane conductance*

| Lipid | Conductance (mho $cm^{-2}$) | |
|---|---|---|
| | + DNP | − DNP |
| DiGluDiGly | $2.0 \times 10^{-7}$ | $4 \times 10^{-9}$ |
| LysPG | $1.2 \times 10^{-7}$ | $6 \times 10^{-9}$ |
| PG | $2.1 \times 10^{-8}$ | $2 \times 10^{-9}$ |

Table 2. *pH-Optimum of membrane conductance in presence of uncouplers*

| Lipid | Uncoupler | $pK_a$ of uncoupler | pH-Optimum of conductance |
|---|---|---|---|
| DiGluDiGly | DNP | 4.0 | 3.9–4.1 |
| LysPG | DNP | 4.0 | 2.6–2.9 |
| PG | DNP | 4.0 | – |
| DiGluDiGly | S 13 | 6.8 | 8.3–8.9 |
| LysPG | S 13 | 6.8 | 5.7–6.4 |
| PG | S 13 | 6.8 | 9.4–9.8 |

presence of $9.3 \times 10^{-5}$ M DNP for all three types of bilayers is shown. The pH optimum for the neutral lipid was found at about 4.0 and for the positive LysPG at 2.8. LysPG membranes possessed a 50-times-higher conductance than DiGluDiGly at or below the pH optimum for LysPG. This difference became nil at higher pH. An increase in the conductance of PG bilayers in the presence of $9.3 \times 10^{-5}$ M DNP was not detectable.

Since DNP is routinely used as an uncoupler in the pH range 6 to 8, it was of interest to investigate the effect of lipid charge on conductance induced by this uncoupler at this higher pH value. Membrane conductance was therefore measured for all three lipids at pH 7.0 in the absence and in the presence of $10^{-3}$ M DNP (Table 1). At this concentration, DNP caused a conductance of $2.1 \times 10^{-8}$ mho $cm^{-2}$ in PG bilayers, but it was about 10 and 6 times more effective in DiGluDiGly and LysPG membranes, respectively.

In Table 2, the pH optima of conductance for the three lipids in the presence of DNP and S 13 are summarized.

## Discussion

The results reported in this paper demonstrate that S 13, perhaps the most potent known uncoupler of mitochondrial oxidative phosphorylation,

greatly increases conductance of lipid bilayers. The concentrations employed are comparable to those used in mitochondrial experiments and thus this compound is more effective than any other known uncoupler tested on bilayers. The conductance in the presence of the salicylanilide (as well as DNP) is a function of the aqueous concentration of this compound, the pH of the buffer, and the charge on the lipid in the bilayer.

At a given concentration, the salicylanilide is most effective in increasing the conductance of the positively charged bilayer of LysPG and least effective in that of the negatively charged PG. An intermediate effect of S 13 on conductance is observed on the uncharged DiGluDiGly bilayer. In all three types of membranes, the uncoupler-induced increase in conductance is accompanied by a proton (or hydroxyl ion) potential; i.e., the uncoupler effectively increases the permeability of the bilayer to protons (or hydroxyl ions). A linear relationship between the conductance and the uncoupler concentration was observed for all three types of bilayers.

Two models for uncoupler-induced proton permeability have been considered in detail. Markin *et al.* (1969) regarded the uncoupler molecules as "carriers" of protons in the membrane phase. In this model, the membrane is assumed to contain a certain concentration of both species of the uncoupler, i.e., the neutral undissociated form and the anionic form. Association and dissociation of protons (derived from the aqueous phase) with uncoupler takes place at both interfaces. Net proton translocation is achieved by the following steps: (1) association of protons with uncoupler anions at one interface; (2) diffusion across the membrane in the form of the protonated species; (3) dissociation at the other interface; and (4) movement of uncoupler anion back to the first interface, thus completing a cycle. The charge is carried by protons at both interfaces and by uncoupler anions within the membrane. Movement of uncoupler molecules across interfaces is considered to be slow compared to that of protons, and thus the magnitude of conductance is determined by the concentration of that form of uncoupler which is lowest in the membrane, assuming equal mobility for both.

Lea and Croghan (1969) and recently Finkelstein (1970) have proposed an alternative mechanism: the membrane is assumed to be permeable only toward an "ion complex" of the form $HU_2^-$, where $U^-$ is the uncoupler anion and H a proton. This charged complex is considered to be present at low concentrations in the aqueous phase, and conductance, in the limit of zero current, depends on the concentration of this complex in the membrane.

The two mechanisms differ with respect to the predicted effect of uncoupler concentration on conductance. In the latter "ion complex" mecha-

nism of Lea and Croghan (1969), conductance varies with the square of the uncoupler concentration, whereas in the former "carrier" mechanism it depends linearly on uncoupler concentration. Clearly the experimental findings reported here are only consistent with the "carrier" mechanism since the conductance (at fixed pH) was found to depend on the first power of the concentration of uncoupler.

Although the two models ("ion complex" and "carrier") predict a pH of maximum conductance at constant uncoupler concentration, they differ in the dependence of this pH on the pK of the uncoupler. If the "ion complex" is the charge-carrying species in the membrane, the pH maximum must occur at the pK of the uncoupler because the aqueous concentration of "ion complex" is highest at this pH. In the "carrier" mechanism, the pH of maximum conductance depends not only on the pK of the uncoupler but also on the relative membrane solubility and mobility of the undissociated and dissociated forms. Previously published work is in disagreement about the pH of maximum conductance for several uncouplers. Liberman and Topaly (1968) reported the pH maxima at the pK for derivatives of carbonylcyanide phenylhydrazone, whereas in this laboratory (Hopfer *et al.*, 1968) the pH maxima were found above the pK. This could be explained by differences in the experimental conditions in the two laboratories, or by the fact that in both cases the aqueous concentration of uncoupler was assumed to be constant at all pH values.

The two mechanisms also differ with respect to the effect that a variation in lipid charge will have on conductance. Modifications of lipid bilayer charge would be expected to affect the concentrations of all mobile ions in the boundary region and in the membrane phase. Compared to a neutral lipid bilayer, a positively charged bilayer will increase, and a negatively charged bilayer will decrease the concentrations of both $U^-$ and $HU_2^-$ in the membrane at constant aqueous uncoupler concentrations in the aqueous uncoupler concentrations in the aqueous phase. However, whether an increase or decrease of anion or "ion complex" yields a corresponding change in conductance depends on the mechanism of charge (proton) translocation. In the model of Lea and Croghan (1969), conductance is determined at all pH values by the concentration of the "ion complex" so that a variation in lipid charge is expected to alter conductance by the same factor over the whole pH range. In contrast, the "carrier" mechanism predicts that bilayer conductance depends on the membrane concentration of undissociated uncoupler above the pH maximum and on that of the anion below it. Modification of lipid charge with its influence only on anion concentration of the membrane is expected to shift the pH at which the

maximum of conductance is observed; it will be lower for the positive LysPG and higher for the negative PG compared to neutral DiGluDiGly. At the pH below the maximum for LysPG, the uncoupler anion limits conductance in all three differently charged membranes; changes in the level of anion in the membrane caused by the use of differently charged lipids therefore change the conductance. Similarly, the amount of undissociated uncoupler is the limiting factor in conductance in all three types of bilayers above the pH maximum of PG, so that despite the difference in lipid charge no differences in conductance are expected. In the pH range between the optimum for DiGluDiGly and that for PG, the protonated form restricts conductance in LysPG and DiGluDiGly, but the anion limits it in PG membranes. Therefore, in this pH range at equal aqueous uncoupler concentration, conductance is expected to be the same for DiGluDiGly and LysPG, but lower for PG bilayers. The data presented in Figs. 2 and 4 and Table 1 support the "carrier" model, but are in conflict with the "ion complex" model, since variation in lipid charge does not alter conductance by a constant factor over the whole pH range, but produces a shift in the pH of maximum conductance.

Assuming that the hydrophobic part of the bilayer is equal in all three types of membranes and that the difference in conductance is due to an electrostatic contribution of the fixed lipid charge, then under conditions where conductance is a function of uncoupler anion concentration, the observed conductance ratios are related to the difference in potential between the membranes of different lipids by the following equation (*see* Appendix for derivation):

$$\Psi^{\mathrm{DiGluDiGly}} - \Psi^{\mathrm{LysPG}} = \frac{RT}{F} \ln \frac{G_0^{\mathrm{DiGluDiGly}}}{G_0^{\mathrm{LysPG}}}$$

where $\Psi$ = potential, $\frac{RT}{F}$ = 25.7 mV at 25° C, and $G_0$ = conductance in the limit of zero current. As shown in Fig. 1, the measured conductance (with an impressed potential of $\pm 20$ mV) is the same as $G_0$. The potential difference calculated in this manner from the conductance ratio is about 100 mV more positive for LysPG and 60 mV more negative for PG than for DiGluDiGly.

The results on these model bilayer membranes indicate that variation in lipid charge can cause significant differences in the magnitude of proton conductance induced by uncouplers. The results of the experiments with DNP suggest that these differences are sufficient in magnitude to explain the observed differences in efficacy of these compounds in uncoupling oxidative phosphorylation in mitochondria as compared to many bacteria.

For example, although the inner membrane of rat liver mitochondria and the protoplast membrane of *Micrococcus lysodeikticus* both contain about 25% lipid (Korn, 1969; Gilby, Few & McQuillen, 1958), the composition of the lipids differs considerably. The mitochondrial membrane contains 25% acidic and 75% uncharged lipids (Stoffel & Schiefer, 1968; M. Guarnieri, B. Stechmiller & A. L. Lehninger, *in preparation*), whereas the membrane of *M. lysodeikticus* contains 82% acidic and only 18% neutral lipids (Macfarlane, 1961). Perhaps the finding that the concentration of DNP needed for uncoupling is approximately 10 times higher in *M. lysodeikticus* than in mitochondria (Mitchell, 1961; Gel'man *et al.*, 1967) is explained by the higher acidic lipid content of the bacteria. Certainly, further experimentation will be required to test this idea. However, it is clear from the results of this study, and our previous study on the effect of variation of lipid charge on ion permeability (Hopfer *et al.*, 1970), that the charge on lipids may play an important role in controlling the physical properties of natural membranes.

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## Appendix

### *Relation of Fixed Lipid Charge to Conductance Induced by Uncouplers*

Fixed lipid charges change the electrostatic potential of the membrane with respect to the bulk aqueous phase. The concentrations of mobile ions are influenced by this potential. Therefore, at fixed pH and uncoupler concentration:

$$a_{U-}^{\text{LysPG}} = k_{U-}\, a_{U-}^{W} \exp \frac{zF(\Psi^{W} - \Psi^{\text{LysPG}})}{RT} \tag{1}$$

and

$$a_{U-}^{\text{DiGluDiGly}} = k_{U-}\, a_{U-}^{W} \exp \frac{zF(\Psi^{W} - \Psi^{\text{DiGluDiGly}})}{RT}, \tag{2}$$

where $a_{U-}$ = activity of uncoupler anion, $k_{U-}$ = its partition coefficient between membrane and aqueous phase, $z$ = charge of the anion, F = Faraday equivalent, $R$ = gas constant, $T$ = absolute temperature, $\Psi$ = potential, and the superscripts refer to the water ($W$) or membrane phases, respectively.

Dividing Eq. (1) by Eq. (2) results in the following relationship at equal uncoupler anion concentration in the aqueous phase:

$$\frac{a_{U-}^{\text{LysPG}}}{a_{U-}^{\text{DiGluDiGly}}} = \exp \frac{zF(\Psi^{\text{DiGluDiGly}} - \Psi^{\text{LysPG}})}{RT}. \tag{3}$$

Since, in the case of LysPG, conductance below the pH optimum is a function of uncoupler anion concentration in the membrane, the following relationship ($G_0$ = con-

ductance in the limit of zero current) may be derived:

$$\frac{G_0^{\text{LysPG}}}{G_0^{\text{DiGluDiGly}}} = \frac{a_{U-}^{\text{LysPG}}}{a_{U-}^{\text{DiGluDiGly}}} = \exp \frac{zF(\psi^{\text{DiGluDiGly}} - \psi^{\text{LysPG}})}{RT}. \quad (4)$$

Rearrangement of this relationship and introduction of $z = -1$ yields:

$$\psi^{\text{DiGluDiGly}} - \psi^{\text{LysPG}} = \frac{RT}{F} \ln \frac{G_0^{\text{DiGluDiGly}}}{G_0^{\text{LysPG}}}. \quad (5)$$

Similar equations can be written for PG membranes.

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