BBA 75202

PROPERTIES OF BLACK LIPID MEMBRANES OF CHLOROPLAST PIGMENTS

HIE PING TING, WILLIAM A. HUEMOELLER, SUBBIAH LALITHA, A. LOUISE DIANA AND H. T. TIEN

Department of Biophysics, Michigan State University, East Lansing, Mich. (U.S.A.)

(Received July 23rd, 1968)

SUMMARY

1. Physical properties of black lipid membranes formed from chlorophylls and chloroplast pigments in aq. NaCl solutions have been determined. The properties of chlorophyll black lipid membranes show significant differences from those black lipid membranes produced from phospholipids, synthetic surfactants, or oxidized cholesterol.

2. The chlorophyll black lipid membranes as measured by a reflectance technique have a thickness of about 105 ± 5 Å. Possible sources of error in assessing the membrane thickness are discussed.

3. The bifacial tension of the chlorophyll black lipid membranes in 0.1 M NaCl, as measured by the bulging method, was found to be 3.8–4.5 dynes/cm.

4. The permeability to water of chlorophyll black lipid membranes has been determined by an osmotic flow method. NaCl was used as the solute. A value 51 μ/sec has been obtained for the permeability coefficient. The effect of temperature on water permeability has also been investigated. The Arrhenius activation energy has been found to be 4.2 kcal/mole.

5. Electrical parameters of chlorophyll black lipid membranes have been measured in various NaCl solutions (10^{-3} to 1 M). In dilute solutions ($10^{-2.5}$ M or less) the d.c. resistance was $1 \cdot 10^6$–$3 \cdot 10^6$ $\Omega \cdot cm^2$. At higher concentrations, the resistance of the membrane was lower by a factor of ten. The temperature dependence of membrane resistance has been measured. A value 15.9 ± 0.3 kcal/mole has been obtained for the activation energy.

6. It is concluded that black lipid membranes produced from chloroplast pigments are useful model systems for further investigation which may give insight into the processes relevant to photosynthesis.

INTRODUCTION

As a result of studies made by the monolayer techniques and the classical permeability measurements, the well known bimolecular or bilayer lipid model for the plasma membrane has been developed[1]. This simple and elegant model first suggested by GORTER AND GRENDEL[2] has been seemingly supported by a vast number of investigations using sophisticated methods such as the X-ray diffraction analysis and

electron microscopy. It has now become evident that membranes of less than 100 Å thick are present, not only in the plasma membrane, but also in membranes of bacteria, mitochondria, retina, nerve myelin and chloroplasts*. In all these systems it has been postulated that a highly organized structure of lipids in a lamellar arrangement as the central core exists in all membranes. Further evidence in support of the bilayer lipid model was provided by the formation of black lipid membranes as an isolated structure in aqueous solution[3,4]. A review of the work on black lipid membranes has been given recently by TIEN AND DIANA[5].

In the case of chloroplasts, CALVIN[6] has suggested that the lamellar form of the photosynthetic pigments is essential to the functioning of the photochemical apparatus. With this view in mind, the present work was undertaken to investigate (i) whether a black lipid membrane could be constituted from photoactive pigments, and (ii) the possibility of using such a black lipid membrane system as energy transducers in the study of certain aspects of photophysical and photochemical processes. This paper reports the properties of black lipid membranes produced from chloroplast pigments, which hitherto have not been studied. The interaction of light and the effect of chemical agents on these new black lipid membranes will be described in separate publications[7,8].

EXPERIMENTAL

Materials

All reagents were c.p. grade and were used as obtained. The laboratory distilled water was re-distilled in an all-glass apparatus before use. The solvents used in this work were methanol, light petroleum (b.p. 30–60°), various *n*-alkanes (C_6 to C_{12}, 99 mole%, Phillips Petroleum Co.). These solvents were used without further purification. Fresh spinach leaves obtained from local markets, which had been processed (*i.e.* washed and with rootes removed), were used. Chlorophylls and xanthophylls purchased from commercial sources were also tried (K and K Rare Chemicals, and Sigma Chemical Co.).

Preparation of solutions

Unless otherwise stated all black lipid membranes studied were formed in unbuffered 0.1 M NaCl. The black lipid membrane-forming solutions were prepared by procedures described elsewhere[9]. In brief, the basic extraction procedure was as follows: (i) fresh spinach leaves free of midribs were boiled for 1 min in distilled water, (ii) the water was then cooled rapidly with ice and decanted, (iii) the leaves were pressed and dried between layers of paper towels, (iv) the dried leaves were then extracted 3 times with light petroleum–methanol (2:1, v/v) at room temperature using a procedure given by STRAIN AND SVEC[10], (v) the combined extracts were evaporated to dryness in a flash evaporator (Buchler Instruments Co.). The residue thus obtained was completely soluble in alkanes and was used for black lipid membrane formation. In later experiments it was found that the boiling step described in

* References may be found in a number of symposium publications: (a) *Symp. on the Plasma Membrane, Circulation*, 26, part 2 (1962); (b) *Brookhaven Symp. in Biol.*, 19 (1967); (c) *Mitochondrial Structure and Compartmentation*, Adriatica Editrice, Bari, 1967; (d) *Symp. on Lipid Monolayer and Bilayer Models and Cellular Membranes, J. Am. Oil Chemists' Soc.*, 45, No. 4 (1968).

the STRAIN–SVEC procedure was not necessary and therefore was omitted in later preparations. It should be mentioned that black lipid membrane-forming solutions could also be prepared from commercially available pigments. For instance, a black lipid membrane-forming solution used in earlier experiments consisted of 3.5% chlorophyll and 5.2% xanthophyll in *n*-octane (w/v). No apparent differences were observed between the fresh and commercial preparations insofar as the formation of a black lipid membrane was concerned.

Methods of black lipid membrane formation

The two basic methods described earlier were used in black lipid membrane formation[4,5]. For thickness determination by reflectance technique, a Teflon loop of 3 mm (internal diameter) was suspended from a wire inside a glass tube of 8 mm (internal diameter). The glass tube was connected through an outlet at the bottom to a 0.1 M NaCl reservoir. The glass tube was first filled with the aqueous solutions to just below the level of the loop. A thin layer of lipid solution was then introduced onto the surface. By raising the oil–water interface over the loop, a self-thinning film was formed in the process. The film formed in this manner was thick and usually thinned down in a few minutes to the black state. In the experiments of bifacial tension, water permeability, and electrical properties, the black lipid membranes were formed either using the brush technique[4] or the injection method. In the latter case, a 2-μl drop of the lipid solution was shot onto the aperture in the wall of the Teflon chamber. This was accomplished by means of a microsyringe (100 μl) with a repeating dispenser attachment (Model PB-600-1, Hamilton, Whittier, Calif.). This dispenser attachment permitted a minute and an equal quantity of lipid solution to be ejected onto the opening each time the release button was actuated. It should be mentioned that the formation of a black lipid membrane is basically a very simple process. However, a certain amount of practice and experience are necessary. The basic techniques and precautions have been given in earlier publications[4,5].

Apparatus and procedure

Since a number of physical properties of these new black lipid membranes were characterized in this work employing quite different experimental set-ups, the apparatus and procedure used are described in the following paragraphs under separate headings.

Thickness of chlorophyll black lipid membranes

The reflectance of the black lipid membranes constituted from chloroplast pigments was measured with an optical set-up described previously[11]. Briefly, a high intensity light source was focused on the membrane and the intensity of the reflected light was measured with a photometer (Photomultiplier Microphotometer, American Instrument Co.). The resulting intensities of the reflected light from the silvery-golden and black films were recorded. The Brewster angle of these black lipid membranes was not determined. However, the reflectance of the membrane was measured at 5461 Å and 4350 Å.

Bifacial tension of chlorophyll black lipid membranes

The bifacial tension (free energy) of the black lipid membranes made from chloroplast pigments was measured with the apparatus used earlier in similar studies[12,31].

The apparatus consisted of a Teflon sleeve held between a set of ground-glass joints. The inner chamber was connected to an infusion-withdrawal pump *via* a ballast chamber. The outer chamber was connected to another ballast chamber (for a detailed diagram, see Fig. 4, ref. 12). The infusion-withdrawal pump was usually started after the observable part of the membrane had become completely black for 2 min. The infusion of the aqueous solution made the membrane bulge out and the maximum pressure difference was noted when the black lipid membrane was hemispherical in shape. The bifacial tension was calculated using the formula $\gamma = Pd/8$, where P is the pressure difference across the black lipid membrane and d is the diameter of the aperture.

Water permeability of chlorophyll black lipid membranes

The apparatus and procedure for osmotic water permeability studies were the same as those previously reported[13]. The cell assembly consisted of a closed inner compartment which was immersed in a container of much larger volume. The inner compartment was connected to a micrometer syringe of 0.25 ml (Cole-Palmer Instrument and Equipment Co., Chicago, Ill.) the volume of which could be adjusted accurately to about 0.01 μl. The temperature of the whole cell assembly could be maintained within about 0.05° by flowing thermostated water through a coil placed in the outer compartment. After the film had become black, a known quantity of conc. NaCl solution was added to the outer compartment and thoroughly mixed. The difference in osmotic pressure across the black lipid membrane caused the efflux of water and this was manifested by the inward bulging of the membrane. By adjusting the micrometer dial, the volume change as a result of water efflux could be measured. The setting of the dial was based upon the visual observation of pattern of light reflected from the black lipid membrane. The water permeation studies were carried out in the dark except the black lipid membrane was observed with light transmitted through a narrow-band interference filter (5461 Å). The temperature dependence of water permeability was also investigated.

Electrical properties of chlorophyll black lipid membranes

The electrical properties of the chlorophyll black lipid membrane examined included the d.c. resistance, dielectric breakdown, and current–voltage relationship. The black lipid membrane resistance was also measured as a function of temperature, and the I-V curves were obtained by measuring membrane potential as a function of applied voltage. The measurements were performed in various NaCl solutions ranging from 10^{-3} to 1.0 M. The temperature dependence of the d.c. resistance was studied in an apparatus reported earlier[14] except that a simple box containing a set of fixed resistors (10^5 to 10^{10} Ω) and a voltage source (Hg battery with a precision potentiometer) was used. As mentioned before, black lipid membranes were formed on a Teflon sleeve separating two aqueous solutions. Calomel electrodes with saturated KCl bridges were used. The I-V curves of the membrane were obtained with a set-up consisting of an inexpensive electrometer (Model EUW-301, Heath Co.) and a pH millivolt test box (Model EUA-20-12, Heath Co.). The circuit diagram for this simple set-up is identical to the one given earlier (Fig. 2, ref. 14).

RESULTS AND DISCUSSION

General considerations

The black lipid membranes constituted from spinach chloroplast pigments characterized in this study show significant differences from those of "ordinary" black lipid membranes formed from materials such as phospholipids, synthetic surfactants and oxidized cholesterol[5]. Before discussing the results of this work certain important features of the black lipid membrane as model systems for the natural membrane may be mentioned. In particular, we would like to compare the organization of the black lipid membrane with that of postulated chloroplast structures.

Evidence derived from electron microscopy[15], X-ray diffraction[16], and birefringence[17] as well as circular dichroism[18] studies strongly indicates that the photosynthetic apparatus is composed of highly organized lamellar structures[19]. For instance, a simple model of a chloroplast thylakoid is pictured by MENKE[20] to consist of two protein layers separated by a Gorter-Grendel bilayer of lipids. Other models have been proposed by WEIER AND BENSON[21] and by MÜHLETHALER[22]. The salient features in all these models lies in their oriented lipid core onto which other important cellular constituents such as proteins may interact through either ionic or Van der Waals attraction or both. Within the chloroplast, the usual picture is that a granum is composed of a stack of disc membranes. Each disc membrane is believed to separate two phases (aqueous?) forming the so-called inner and outer spaces[23]. The thickness of this disc membrane is estimated to be about 70–120 Å (refs. 20, 22). It is in this context that a black lipid membrane (usually less than 90 Å thick) separating two aqueous solutions provides a useful model system for investigation.

As has been discussed elsewhere[24], a black lipid membrane possesses two aqueous solution–membrane interfaces or a *biface* (this term is introduced to stress the two co-existing oil–water or membrane–solution interfaces). This biface, in the order of molecular dimension, is the site where the interfacial-active molecules are located. From the well-known principles of interfacial chemistry, it is to be expected that the constituent molecules of the biface must be oriented in such a fashion that their mutual interaction energy is at a maximum. Although the macroscopic concepts such as dielectric constant and refractive index have been applied to the biface, it must be admitted that much basic physical chemical investigation is necessary in order to achieve a better understanding of the black lipid membrane systems.

The thickness of chlorophyll black lipid membranes

Using previously described optical techniques, we have made a preliminary estimation of the thickness of the membrane. The results given in Table I are based upon black lipid membranes produced by chloroplast lipid solution at $22 \pm 1^{\circ}$. Although the refractive indices of these membranes are not available, the calculated thickness values using assumed indices of refraction at two different wavelengths are in excellent agreement. The results imply that the indices of refraction of the membranes (in the silvery-golden and in the black state) change negligibly with wavelength of light used. It should be pointed out, however, that the thickness of the chlorophyll black lipid membrane reported here might differ from the true value by as much as 50 % owing to the use of assumed refractive index values. The other source of error in assessing the thickness of black lipid membrane based upon the simplified Rayleigh

TABLE I

RESULTS OF REFLECTANCE MEASUREMENTS AND CALCULATED VALUES FOR THE THICKNESS OF THE BLACK MEMBRANE

I_b and I_s are the intensities of reflected light from the black and silvery films, respectively. Aqueous solution: 0.1 M NaCl.

λ (Å)	*Relative intensity*, I_b/I_s	*Thickness* (Å)
4350	0.126, 0.167	105 ± 5*
5460	0.0572, 0.0616	105 ± 5*

* These values could be in error by as much as 50%. See DISCUSSION.

equation is the assumption that the black lipid membrane is a homogeneous and isotropic structure characterized by a single refractive index. As discussed elsewhere[24], a more realistic model may be represented by a triple-layered structure with different refractive indices for each layer. Therefore, the thickness of the membrane will be about 10% larger than the value calculated according to Rayleigh's equation.

In spite of the afore-mentioned uncertainties, we believe it is useful to suggest a structural model to account for the calculated results. This is shown schematically in Fig. 1. Several orientations of the porphyrin head group at the biface are possible. Since the interior of the black lipid membrane is believed to be liquid-like, the porphyrin group may be in a "dynamic" state. That is, the porphyrin plate may lie sometimes parallel to the biface, sometimes perpendicular and at other times somewhere in between. However, a more or less perpendicular orientation is favored based upon the bifacial tension data (see following section). With this picture in mind, the observed thickness of the membrane could be accounted for by the depth of anchoring of the phytyl chain and by the amount of other lipids such as carotene located in the interior of the membrane (see Fig. 1).

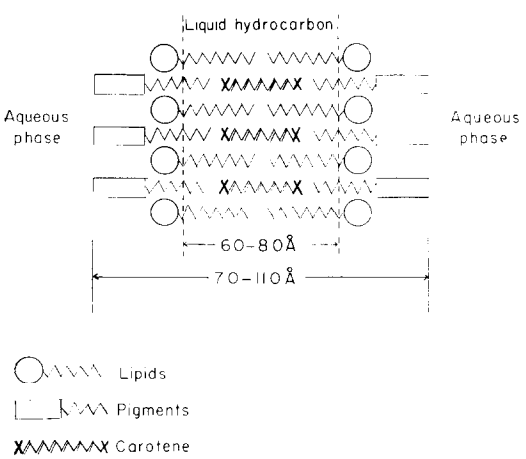

Fig. 1. Schematic model of possible molecular organization for a black lipid membrane of chloroplast pigments. The membrane reported here was formed on a Teflon support separating two aqueous solutions. When formed in this manner, the transverse electrical and transport properties of the membrane can be measured and controlled chemical investigation can be carried out. The thickness of the membrane was about 100 Å (see text).

The bifacial tensions of the chlorophyll black lipid membrane

The bifacial tensions of the black lipid membrane formed from chloroplast pigments in *n*-octane and in *n*-octane–butanol mixture are, respectively, 4.5 ± 0.1 and 3.8 ± 0.2 (dynes/cm). The results are significantly higher than those of other black lipid membranes produced from natural lipids[24]. It appears also that the pigment solvent used has a noticeable effect on the results. Whether or not the solvent molecules are incorporated into the black lipid membrane structure we are not certain. The lower value in the case of the octane–butanol mixture strongly suggests however that the bifacial region is modified owing to the presence of additional interfacial-active species.

Extending the equations and reasonings used in the monolayer studies at the air–water interface to the bilayer lipid membranes at the water–oil–water biface[14], the bifacial pressure to which the constituent pigments (and other interfacial-active species) are subjected is given by

$$\pi_i = \gamma_0 - \gamma_i \qquad (1)$$

where π_i is the bifacial pressure, γ_0 and γ_i are the interfacial tension between liquid hydrocarbon solvent and water and the bifacial tension of the black lipid membrane, respectively. Hence, the bifacial pressure is of the order of 45–50 dynes/cm. Therefore, at the observed bifacial pressure one would expect that the pigment molecules (as typified by chlorophylls) in the membrane should occupy their limiting areas. In other words, the molecules in the black lipid membrane are closely-packed. On the basis of the bifacial tension data, it seems likely therefore that the porphyrin plates of the molecules are oriented more or less perpendicularly to the biface as is illustrated in Fig. 1.

The permeability to water

The osmotic water permeability coefficient, P_0, is calculated using the equation

$$P_0 = 0.925\, RTP' \qquad (2)$$

where $P' = J/\Delta\pi$, R and T have the usual significance. J is the net volume flow of water in time dt and $\Delta\pi$ is the osmotic pressure difference across the black lipid membrane. The conversion factor (0.925) is used to express P_0 in μ/sec units. The volume change as a function of time for a typical run is shown in Fig. 2. Results of a number of experiments using black lipid membranes generated from several different lipid solutions are summarized in Table II.

TABLE II

VALUES OF P_0 FOR BLACK LIPID MEMBRANES PRODUCED FROM FOUR DIFFERENT LIPID SOLUTIONS AT 22 ± 1°

Chlorophyll black lipid membranes formed from	*Permeability coefficient, P_0 (μ/sec)*
Chlorophyll–xanthophyll mixture in *n*-octane*	51.3
Chloroplast pigments in *n*-octane**	51.4
Methanol–light petroleum extract in *n*-octane (no boiling)**	48.5
Methanol–light petroleum extract in octane–butanol mixture**	51.7

* Obtained from K and K Chemical Co.
** See ref. 10 and EXPERIMENTAL.

In contrast to our earlier findings with black lipid membranes produced from oxidized cholesterol, the osmotic permeability coefficient for chlorophyll black lipid membranes is about 6 times larger[13]. The effect of temperature on permeability is shown in Fig. 3. In this typical Arrhenius plot (log P_0 *vs.* $1/T$), a straight line may be interpreted to mean that a change in membrane structure with temperature (in the temperature examined) is perhaps not involved. Also there is little evidence to suggest that a change in the mechanism of water permeation has taken place with temperature. The activation energy for permeation of water is found to be 4.2 kcal/mole in the range 17.5–34.0°.

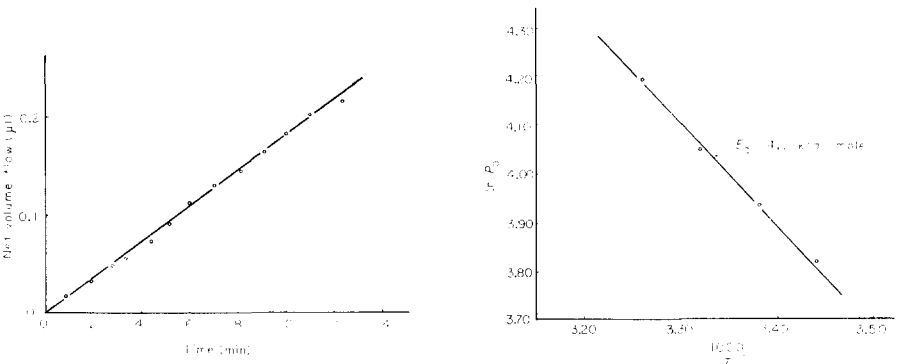

Fig. 2. Volume flow of water across a chlorophyll black lipid membrane (about 100 Å thick) as a function of time. The solute used was NaCl; temp., 22 ± 1°.

Fig. 3. Effect of temperature on water permeability of a black lipid membrane of chloroplast pigments. The ordinate is plotted as the natural log of osmotic permeability coefficients.

At present, it is difficult to make any meaningful interpretation concerning the relatively high permeability to water by the chlorophyll black lipid membrane ($51 \cdot 10^{-4}$ cm/sec). As far as we are aware, no data exist for the thylakoid membranes (comparable to chlorophyll black lipid membrane) although a number of investigations have been made on the permeability and osmotic properties of the chloroplast membrane[25–30].

The electrical properties of chlorophyll black lipid membranes

The d.c. resistance of the black membranes formed from chloroplast pigments was measured in various NaCl solutions (10^{-3}–1 M) and as a function of applied voltage. Membranes in 10^{-3}–$10^{-2.5}$ M NaCl solution had resistances of $1 \cdot 10^6$–$3 \cdot 10^6$ $\Omega \cdot cm^2$ and the observed resistance of the membrane exhibited a strong concentration dependence on the bathing medium. However, the membrane resistances were one order of magnitude lower in 10^{-2} M and higher concentrations of NaCl but did not show any marked concentration effect. The results of a number of measurements are presented in Fig. 4. Within the applied voltages used (10–150 mV), the membrane resistance was ohmic and irrespective of NaCl concentrations (see Fig. 5). It is interesting to note that ordinary black lipid membranes in 0.1 M electrolyte solution (such as NaCl) have resistances about 2–3 orders of magnitude higher than the chlorophyll black lipid membranes. This difference in resistance may be owing to the following factors: (i) the chlorophyll black lipid membrane can dissolve greater amount of ions,

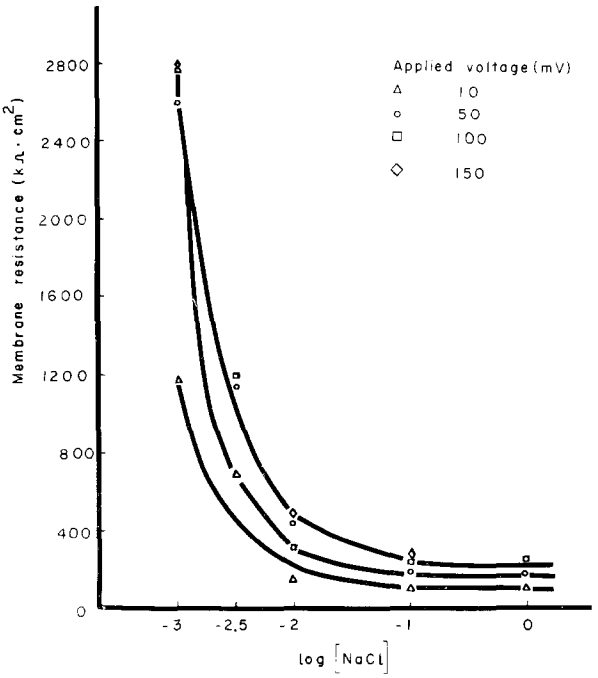

Fig. 4. Plots of membrane resistance as a function of NaCl concentration bathing the membrane at four different applied voltages. Temp., 22 ± 1°.

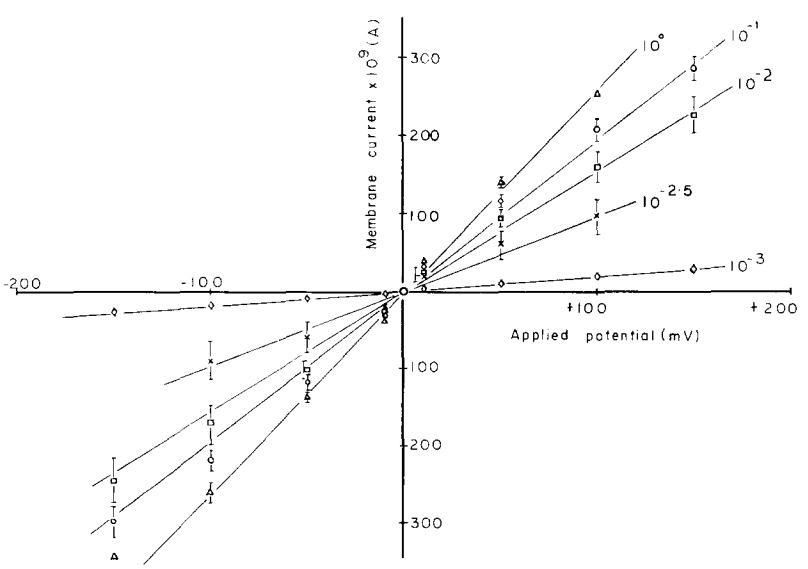

Fig. 5. Linear plots of the current–voltage characteristics of chloroplast black lipid membranes in various NaCl solutions. Temp., 22 ± 1°. The ordinate is plotted as membrane current per cm² of area.

(ii) the constituent molecules in the chlorophyll black lipid membranes can interact more strongly with the ions in the bathing medium resulting in a greater number of ions at the biface, and (iii) the chlorophyll black lipid membranes are intrinsically more conductive. The first two factors are self-explanatory for in either case more charge carriers (ions) are available in (and near) the membrane for conduction. The third factor is less obvious and deserves a brief elaboration. As has been shown by TIEN[7,8], the chlorophyll black lipid membranes exhibit both photovoltaic effect and photoconductivity. These photoelectric phenomena indicate implicitly that charge carriers are generated upon illumination of the membrane. It may be argued that, in the absence of photoactive light, mobile charge carriers could be also produced thermally and/or by the external field. This could account for the observed lower resistance of the chlorophyll black lipid membranes. It should be mentioned that little is known about the exact mechanism for charge transport in this type of membrane. Experiments are now in progress to obtain the needed information on both the mechanism of charge generation and conduction.

The dielectric breakdown voltage of chlorophyll black lipid membranes was determined by the method previously reported[11]. If the applied voltages were greater than about 150 mV, the membranes broke within a few seconds. The black lipid membranes formed from commercial pigments had a lower dielectric breakdown strength and usually ruptured when the voltages exceeded about 100 mV. Nevertheless, it should be noted that, for a membrane of about 100 Å in thickness, the applied voltages correspond to the electric field strength in the order of 100 000 V/cm.

When the resistance of the membrane was measured as a function of temperature it was found that the resistance increased with decreasing temperature in the range of 16–30°. Above 34° the membranes formed were stable for only short periods of time; below 16° precipitation occurred in the membrane. Between the temperature range studied, the membrane resistance (R) followed the well known relationship (a form of Arrhenius equation):

$$R = R_0 \exp(-E/RT) \tag{3}$$

Where E is the activation energy and R_0 is a constant. From a plot of measured resistance *versus* the reciprocal of absolute temperature, the quantity E was obtained

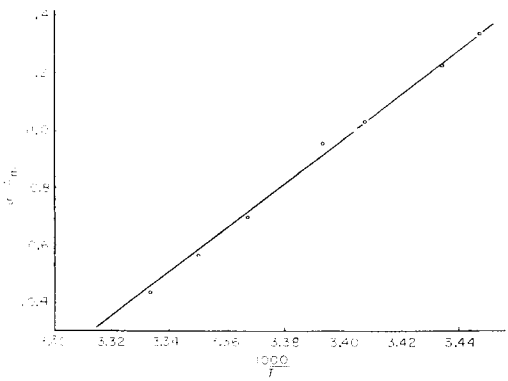

Fig. 6. Temperature dependence of membrane resistance. Activation energy, 15.9 ± 0.3 kcal/mole. The resistance of chlorophyll black lipid membranes was measured in the temperature range of 16–30°.

in the usual manner. A typical plot is shown in Fig. 6. For the chlorophyll black lipid membranes in 0.1 M NaCl, E was found to be 15.9 $\pm$ 0.3 kcal/mole. It should be noted that almost all rate processes observed experimentally can be fitted into a form of Arrhenius equation. Thus, in the absence of additional information, it is difficult to give a unique interpretation of E. In the present case, E may be interpreted to mean either the activation energy required for the charge transport or the creation of charge carriers or both. Further discussion will be deferred until more data are available on the chlorophyll black lipid membrane system.

CONCLUSIONS

This investigation demonstrates that extracts of chloroplasts and photosynthetic pigments such as chlorophylls and xanthophylls, when dissolved in appropriate solvent, can produce black membranes. These black lipid membranes, in the order of bimolecular thickness (about 100 A) are believed to exist in a lamellar form whose structural organization resembles the postulated thylakoid membrane in the chloroplasts. This new experimental structure, when formed at a biface (*i.e.* separating two aqueous solutions), offers new approaches for the study of photophysical and photochemical processes as well as dark reactions that are relevant to photosynthesis.

ACKNOWLEDGEMENTS

This work was aided by grants from the U.S. Public Health Service (GM-14971), National Institute of Health postdoctoral fellowship (GM-37953) to A.L.D., (GM-10890) to H.P.T., and National Institute of Health graduate training grant (GM-01422) to W.A.H.

REFERENCES

1 H. Davson, *Circulation*, 26 (1962) 1022.
2 E. Gorter and F. Grendel, *J. Exptl. Med.*, 41 (1925) 439.
3 P. Mueller, D. O. Rudin, H. T. Tien and W. C. Wescott, *Nature*, 194 (1962) 979.
4 P. Mueller, D. O. Rudin, H. T. Tien and W. C. Wescott, *J. Phys. Chem.*, 67 (1963) 534.
5 H. T. Tien and A. L. Diana, *Chem. Phys. Lipids*, 2 (1968) 55.
6 M. Calvin, *Rev. Mod. Phys.*, 31 (1959) 147.
7 H. T. Tien, *Nature*, 219 (1968) 272.
8 H. T. Tien, *J. Phys. Chem.*, 72 (1968).
9 H. T. Tien, W. A. Huemoeller and H. P. Ting, *Biochem. Biophys. Res. Commun.*, 33 (1968) in the press.
10 H. H. Strain and W. A. Svec, in L. P. Vernon and G. R. Seely, *The Chlorophylls*, Academic Press, New York, 1966, p. 21.
11 H. T. Tien and E. A. Dawidowicz, *J. Colloid Interface Sci.*, 22 (1966) 438.
12 H. T. Tien, *J. Phys. Chem.*, 71 (1967) 3395.
13 H. T. Tien and H. P. Ting, *J. Colloid Interface Sci.*, 27 (1968) 702.
14 H. T. Tien and A. L. Diana, *J. Colloid Interface Sci.*, 24 (1967) 287.
15 R. B. Park and N. G. Pon, *J. Mol. Biol.*, 6 (1963) 105.
16 O. Kratky, W. Menke, A. Sekora, B. Paletta and M. Bischof, *Z. Naturforsch.*, 14b (1959) 309.
17 A. Frey-Wyssling and E. Steinmann, *Biochim. Biophys. Acta*, 2 (1948) 254.
18 E. A. Dratz, A. J. Schultz and K. Sauer, *Brookhaven Symp. Biol.*, 19 (1967) 303.
19 D. Branton, in A. G. Giese, *Photophysiology*, Vol. 3, Academic Press, New York, 1968, p. 197.
20 W. Menke, *Brookhaven Symp. Biol.*, 19 (1967) 328.

21 T. E. WEIER AND A. A. BENSON, in T. W. GOODWIN, *Biochemistry of Chloroplasts*, Academic Press, New York, 1966, p. 91.
22 K. MÜHLETHALER, in T. W. GOODWIN, *Biochemistry of Chloroplasts*, Academic Press, New York, 1966, p. 49.
23 R. SAGER, *Brookhaven Symp. Biol.*, 11 (1959) 101.
24 H. T. TIEN, *J. Gen. Physiol.*, 52 (1968) 125.
25 R. A. DILLEY AND L. P. VERNON, *Arch. Biochem. Biophys.*, 111 (1965) 365.
26 M. ITOH, S. IZAWA AND K. SHIBATA, *Biochim. Biophys. Acta*, 69 (1963) 130.
27 J. DIAMOND AND A. C. SOLOMON, *J. Gen. Physiol.*, 42 (1959) 1105.
28 K. NISHIDA, *Plant Cell Physiol. Tokyo*, 4 (1963) 247.
29 A. D. TOLBERG AND R. I. MACEY, *Biochim. Biophys. Acta*, 109 (1965) 424.
30 L. PACKER, *Ann. N.Y. Acad. Sci.*, 137 (1966) 624.
31 H. T. TIEN, *J. Phys. Chem.*, 72 (1968) 2723.