Symmetric and Asymmetric Bilayers of Retinal

Marvin Rich and Seymour Steven Brody

Department of Biology, New York University

Z. Naturforsch. **33 c**, 735–738 (1978); received April 21/June 13, 1978

Bilayers, Retinal, Vision, Membranes

Asymmetric and symmetric bilayer membrane were formed from monolayers of egg lecithin and various retinal isomers. Capacitance of the asymmetric *all-trans* retinal/lecithin bilayer is 1.2 ± 0.1 μF/cm². Asymmetrically *formed* membranes of 13-*cis* retinal/lecithin and 9-*cis* retinal lecithin were found to be 0.83 ± 0.06 and 0.56 ± 0.05 μF/cm², respectively. Formation of bilayers of the various *cis* isomers appears to require the presence of solvent. Irradiation of the various retinal/lecithin films, under the proper conditions, leads to bilayers with marked changes in capacitance and conductance.

Introduction

Retinal is the chromophore group for all rhodopsin type molecules found in nature (1). Specific retinal isomers are bound in rhodopsin via a protanated Shiff base [2]. While the primary photoreaction is presumably a *cis-trans* photoisomerization [3], Van der Meer, *et al.* [4] has argued from thermodynamic considerations that such a reaction is unlikely because of the "tight fit" of the retinal in opsin.

Interactions between monolayers of retinal isomers and amino acids at an air water interface were reported previously [5–7]. Bilayer studies have been limited to the effects of bright flashes on conductivity [8, 9].

Here we reported on the interactions of various retinal isomers with lecithin in both symmetrically and asymetrically formed bilayers.

Methods and Materials

Bilayers were formed from monolayers as described previously [10, 11]. Two teflon troughs with a volume of about 2 cm³ were used. A thin teflon septum 12.5 μm thick separated the two troughs. A hole 2×10^{-4} cm² was melted into the teflon septum. Capacitance measurements were made by applying a constant voltage pulse, V_i (50 mV, unless otherwise specified) and recording the current transient, i, across the bilayer using a voltage clamp similar to that described by Montal and Mueller [10]. The total capacitance of the membrane, C, was determined from the relationship: $C = \int i dt / V_i$. The specific capacitance of the membrane, C_m, was found by substracting the capacitance of the system from the total capacitance and dividing by the area of the hole in the septum.

The conductance of the membrane was measured with a voltage clamp. The feedback resistance, R_f, of the clamp was of the order of 10^9 ohms. The resistance of the membrane, R_m, was calculated using the relationship:

$$V_0/V_i = R_f/R_m$$

where V_i is the applied voltage and V_0 is the output voltage. The value of R_m is multiplied by the area of the aperture to obtain the resistance as ohm·cm². For illumination experiments an unfiltered, low pressure, mercury arc lamp was focused on the bilayer. The intensity was 5.1×10^4 erg/cm² sec. Bilayers were irradiated for about 15 min unless otherwise noted. Temperature of the solution were about 20 °C. No attempt was made to control the temperature. Assuming the bilayer may be represented as a parallel plate capacitor, thickness d for the membrane were calculated from the relation $C_m = \varepsilon E_0/d$ where E_0 is the permitivity of free space and ε is the dielectric constant of the bilayer material taken to be equal to 2.1 (a long chain of hydrocarbon). The theoretical thickness of the membrane was determined by adding together the lengths of the lecithin molecule 22 Å and the retinal isomers (*all trans*, 15.5 Å; 13-*cis*, 13.5 Å; 9-*cis*, 8.6 Å [6]). All chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.); commercial grade lecithin from egg yolk was used. The spreading solvent for lecithin and retinal was, *n*-hexane. The subphase contained 10 mM KCl.

Results

1) *Bilayers formed asymmetrically:*

All trans-retinal/lecithin bilayers. When lecithin was spread on the aqueous phase of one compartment

Table I. Membranes capacitances, resistances and thicknesses of retinals.

Bilayer (Asymmetric)	# films formed	C_m [μF/cm^2] dark	C_m light	R_m, ohms dark	R_m light	d[Å]* calculated	theoretical d[Å]**
all-trans retinal/lecithin	17	1.2 ± 0.1	1.2 ± 0.1	4×10^9	4×10^9	15.5	37.5
13-*cis* retinal/lecithin	15	0.83 ± 0.6	0.40 ± 0.5	4×10^{10}	7.5×10^9	22.4	35.5
9-*cis* retinal/lecithin	10	0.56 ± 0.5	0.90 ± 0.5	2×10^{11}	3×10^{10}	33.2	30.6
Bilayer (Symmetric)							
lecithin, *all-trans* (symmetric)	4	0.9 ± 0.5		4×10^{10}		20.5	44. ***
lecithin, 13-*cis* (symmetric)	5	0.8 ± 0.1		2×10^{10}		23.1	44. ***
lecithin (symmetric)	28	0.75 ± 0.5		2×10^{10}		24.8	44
lecithin, 9-*cis* (symmetric)	5	0.6 ± 0.1		2×10^{11}		30.8	44. ***
all-trans retinal/*all-trans* retinal		UNSTABLE					

* The calculated thickness was found from the relation $d=\varepsilon E_0/C$ (see text).

** The theoretical thickness is calculated assuming no bending or mixing occurs using values of 22 Å, 15.5 Å, 13.5 Å, and 8.6 Å for the lecithin *all-trans* retinal, 13-*cis* retinal, and 9-*cis* retinal, respectively.

*** These values determined by assuming the dielectric thickness is due to the lecithin molecule. For symmetric bilayers the mole ratio of lecithin to retinal was 1:1.
All bilayers were formed at room temperature in an aqueous phase of 10 mM KCL. Membrane aperture was 2×10^{-4} cm^2.

and *all-trans* retinal on the other, and a few minutes were allowed for the hexane to evaporate bimolecular membranes were readily obtained. The average of 17 membranes had a capacitance of 1.2 ± 0.05 μF/cm^2. These membranes were stable up to several minutes. Illumination had no significant effects on either capacitance or conductance. Breakage and reformation of the irradiated membrane produced a new membrane with no discernable change in either parameter. Conductances were of an order of magnitude higher than those of symmetrically formed membranes and ohmic over a range up to 200 mV. Except for extreme pH values, bilayers are stable and the electrical properties of the membrane are unchanged.

9-cis; 13-cis retinal/lecithin bilayers. Membranes formed by the apposition of a monolayer of lecithin and a monolayer of either 9-*cis* or 13-*cis* retinal had capacitances higher than those of pure lecithin, but lower than those of asymmetric *all-trans* retinal/lecithin membranes (see Table I). Conductances were similar to those observed in lecithin bilayers (Table I). In order to form stable membranes of 9-*cis* retinal/lecithin it was necessary to form bilayers almost immediately after the addition of the lipid solutions to the aqueous phase. Consequently the bilayer contained large amounts of solvent. The capacitances and conductances of these membranes were very low compared with other retinal isomers (Table I). These low capacitances may in part be due to the retention of hexane. Furthermore the large amount of solvent might have promoted mixing between the monolayers.

Breakage and reformation of the 9-*cis* and 13-*cis* membranes did not modify the electrical properties. Upon illumination of these bilayers no changes were observed in the electrical characteristics. If the membrane was broken and reformed after illumination sharp increases in both capacitance and conductance were observed. Repeating this procedure produced sucessive increases in the electrical parameters until a limited value for capacitance were reached (Table I). In addition to increased capacitance after illumination, the reformed membranes did not require the presence of large amounts of solvent during formation.

2) Bilayers formed symmetrically:

Lecithin. When lecithin was spread over both aqueous phases and a couple of minutes were allowed for the solvent to evaporate stable bimolecular membranes could be readily formed. If more than

5 minutes was allowed for the solvent to evaporate, before bilayer formation, it was not possible to form stable membranes. Under such conditions addition of a small amount of hexane to the solvent free monolayers permitted the formation of stable membranes. The capacitance of the bilayer increased as the solvent content of the film decreased.

The average of 28 membranes formed on septas with various apertures $2-6\times10^{-4}$ cm^2 gave capacitances of 0.75 ± 0.05 μF/cm^2. This is in close agreement with the value of 0.72 μf/cm^2 reported by Benz, *et al.* [11]. Conductance measurements varied erratically from membrane to membrane. An average value is given in Table I. Conductances for lecithin membranes were ohmic up to 200 mV. Illumination produced no changes in either capacitance or conductance in the bilayer.

Symmetrically formed retinal/lecithin membranes: Symmetric membranes of equal molar concentrations of all trans retinal or cis retinal and lecithin were found to be stable. Their electrical parameters are given in Table I. Large quantities of solvent were needed to form stable membranes with the cis retinals. Symmetric bilayers could not be formed in the absence of lecithin.

Discussion

Based on the difference between the C_m's for the all trans bilayers formed symmetrically and asymmetrically, it is apparent that asymmetric membranes are formed. On the other hand in the case of 9-*cis* and 13-*cis*, from a comparison of the capacitances for the symmetric and asymmetrically formed bilayers, the possibility of mixing of the monolayers cannot be eliminated.

Dark effects: A membrane thickness of 24.8 Å is calculated from the capacitance measurements for a symmetric lecithin bilayer. This thickness is much less than the length of the hydrocarbon portion of two fully extended phosphatidyl choline chains (44 Å) (Table I). A decrease in thickness may occur if the hydrocarbon chains in the membrane are in a bent state [11].

In the case of all-trans retinal/lecithin bilayers a membrane thickness of only 15.5 Å is calculated. Even assuming the possibility of coiling of the hydrocarbon chains, this value is far below the value of 37.5 Å determined by adding the hydrocarbon lengths of a fully extended phophatidyl choline molecule (22 Å) and that of an all-trans retinal molecule (15 Å). The poor agreement between these thicknesses may, in part, result from the uncertainty as to the correct value of the dielectric constant for the retinals. A plausible explanation for these results might arise from a limited penetration of water between the retinal hydrocarbon chains. This is consistant with the low resistance observed in these membranes. Such a penetration of water presumably does not occur in membranes containing lecithin. The absence of water penetration in films containing lecithin would agree with the lower capacitance and higher resistance observed with symmetric all trans retinal lecithin bilayers.

The lower capacitances of the cis-isomers compared to all-trans may be accounted for, in part, as arising from the retention of solvent in the bilayer. This possibility appears likely because of the large amount of solvent required to form bilayers. Bilayers that retain solvent have capacitances considerably lower than those formed from monolayers, where most of the solvent was allowed to evaporate. Lecithin bilayer membranes formed by the Mueller-Rudin technique vary from $0.3-0.6$ μF/cm^2 depending on the solvent used.

Light effects: The fact that no electrical changes are observed after irradiation is in part understandable for *all-trans* retinal, since under the influence of light a very small percent of all-trans retinal isomerizes to the cis configuration. However no changes are observed upon irradiation of bilayers formed from various cis isomers. Perhaps the compressional forces or bilayer interaction within the membrane may limit the rotational movement needed for isomerization to occur or to allow reorientation of the isomerized molecule. Such forces seem to be absent at the air-water interface where photoisomerization does occur, as evidenced by changes in the electrical parameters of newly formed membranes of irradiated 9-*cis* and 13-*cis* retinal. These newly formed membranes show electrical parameters similar to symmetrically formed *all-trans*-retinal/lecithin membranes indicating isomerization and, possibly, mixing between the phases.

These findings indicate that retinal imparts distinct electrical properties to membranes depending upon it microenvironment or organization.

[1] R. Hubbard, P. K. Brown, and A. Knopf, Nature **133,** 442–446 (1959).
[2] A. Lewis, R. S. Fager, and E. W. Abrahamson, J. Raman Spectr. **1,** 145 (1973).
[3] G. Wald, Science **162,** 230–239 (1968).
[4] K. Van der Meer, J. C. Mulder, and J. Lugthenburg, Photochem. Photobiol. **Vol. 24,** 363–367 (1976).
[5] N. Yckowski and S. S. Brody, Z. Naturforsch. **29 c,** 327–335 (1974).
[6] R. E. Brockman and S. S. Brody, Z. Naturforsch. **26 b,** 119–125 (1971).
[7] S. Hotchandani, R. M. Le Blanc, and F. Boucher, Vis. Res. **15,** 605–611 (1975).
[8] N. Kobamoto and H. T. Tien, Biochim. Biophys. Acta **241,** 129–140 (1971).
[9] M. Schadt, Biochim. Biophys. Acta **323,** 351–366 (1973).
[10] M. Montal and P. Mueller, Proc. Nat. Acad. Sci. USA **69,** 3561–3566 (1972).
[11] R. Benz, O. Frohlich, P. Läuger, and M. Montal, Biochim. Biophys. Acta **394,** 323–334 (1975).