Biochimica et Biophysica Acta, 553 (1979) 1—10

BBA 78350

LIPID AND SALT EFFECTS ON CARBOCYANINE DYE-INDUCED PHOTO-VOLTAGES IN BILAYER MEMBRANES

JOHNNY A. BAKER *, JOHN R. DUCHEK, REBECCA L. HOOPER, RYAN J. KOFTAN and JAY S. HUEBNER

Department of Natural Sciences, University of North Florida, Jacksonville, FL 32216 (U.S.A.)

(Received September 6th, 1978)

Key words: Carbocyanine dye; Cyanine dye; Photo-voltage change; (Bilayer membrane)

Summary

Voltage transients induced by flash illumination of bilayer membranes with sorbed dye, 3,3′-dimethyl-2,2′-oxacarbocyanine chloride (diO-C_1-3-Cl), vary with membrane lipid composition and aqueous solution salt concentration. The voltage transients are probably induced by physical movements of sorbed dye molecules following photo-isomerization. Increased salt (NaCl and NaBr) concentrations in the aqueous solutions reduced the photo-voltage amplitudes by reducing the amount of dye sorbed to the membranes, and by decreasing the effective membrane thickness. The photo-voltage risetimes and falltimes varied systematically with salt concentration and membrane lipid composition, reflecting structural changes in the membrane's surface layer.

Introduction

Cyanine and related dyes are finding widespread usage for photometrically monitoring transmembrane potentials in a variety of artificial and biological membranes. Recent examples include studying membranes of ascites tumor cells [1,2], bacterial cells [3—5], heart muscle cells [6], invertebrate neurons [7], mitochondria [8], red blood cells [9], and vesicles (suspensions) of phosphatidylcholine [10]. Little is known about the interactions of cyanine dyes with membranes. The present work provides data on one carbocyanine dye (Fig. 1) in bilayer membranes containing cholesterol, glycerol monoolein,

* Present address: College of Medicine, University of South Florida, Tampa, FL 33612, U.S.A.

Abbreviations: diO-C_1-3-Cl, benzoxazolinium-3-methyl-2-[-3-(3-methyl-2(3H)-benzoxazolylidene)-1-propenyl] chloride; diO-C_1-3-I, the same as diO-C_1-3-Cl except change chloride to iodide; diO-C_1-3-X, the same as diO-C_1-3-Cl except change chloride to halide; diO-C_5-3-I, benzoxazolinium-3-(1-pentyl)-2(3H)-benzoxazolidene)-1-propenyl] iodide; diO-C_2-3-I, benzoxazolinium-3-ethyl-2-[-3-(3-ethyl-2(3H)-benzoxazolylidene)-1-propenyl] iodide.

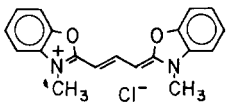

Fig. 1. The structure of the dye diO-C_1-3-Cl in the 'all-*trans*' configuration. The structure of the dye diO-C_5-3-I is obtained by replacing the two methyl groups with *n*-pentyl groups and Cl^- with I^-.

oxidized cholesterol, phosphatidylcholine, and phosphatidylethanolamine in solutions containing NaCl and NaBr.

Photo-electric effects resulting from cyanine dyes have been reported by Ullrich and Kuhn [11,12] and Huebner [13,14]. These effects probably result from light-induced isomerization of membrane-sorbed dye, which causes the charged dye to move toward the adjacent aqueous solution [13,14]. This produces positive photo-voltages when the dye is only on the side of the membrane in contact with the positive electrometer electrode.

The possibility that cyanine dyes may induce these effects by anion motion has been discounted because, (i) experimental results, described below, show that increasing the salt concentration five orders of magnitude reduces the photo-voltage amplitude about one order of magnitude, while making only small modifications in the risetimes, (ii) the risetimes are of the order of magnitude of exit times for pyrene and anthracene from lipid micells, but four orders of magnitude slower than the time required for inorganic ions to move between the solution and lipid micells [15,16], and (iii) the risetimes do not depend upon the mass of the anions.

Materials and Methods

The bilayer membranes were prepared by the syringe method, using membrane forming solutions of 10 mg lipid per ml of decane, in apparatus described elsewhere [17]. 10 min after a membrane had gone black, dye was added to one side of the membrane using a repeating dispenser and a dye stock solution that had been previously prepared and stored frozen. 10 min later the photo-voltages were recorded. These measurements were repeated on three different membranes for each lipid and salt concentration reported below.

The cholesterol was purified by procedure B of Fieser [18]. Oxidized cholesterol was prepared by the method of Tien et al. [19]. The glycerol monoolein was Supelco, 4-4102; the phosphatidylcholine was Sigma, III-E; the phosphatidylethanolamine was Sigma, V. The membrane-forming solutions were prepared in nitrogen and stored below 0° C in nitrogen. Fresh aliquots were used daily, with unused portions being discarded daily. The membrane measurements were made in air at approx. 23° C.

Bridges (to be described elsewhere) were used on the calomel electrodes to prevent electrode salt from contaminating the aqueous solutions. The amount of diO-C_1-3-I which sorbs to bilayer membranes was estimated by preparing lipid suspensions, adding 10 μM dye, filtering the lipid and sorbed dye out by passing the suspension through a dialysis membrane, and measuring the amount of dye remaining in the filtrate photometrically. The filtrate from lipid suspensions with no dye did not absorb in the region of the spectrum near the principle dye absorption peak (482 nm), although absorption toward the blue end of the

spectrum indicated that some lipid passed through the dialysis membrane. The lipid was phosphatidylcholine, (Sigma, IX-E), suspended at 2 mg/ml by hand agitation and magnetic stirring. The suspension was pressed through dialysis membranes (Curtin, 077-032) at 3 atm using a microsyringe filter holder (Millipor, XX30 025). These membranes were prepared by sequentially boiling the membrane for 30 min in 10^{-1} M $NaHCO_3$, redistilled water, 0.02 M ethylaminediamine tetraacetic acid (EDTA) solution at pH 9, twice in distilled water and finally by soaking for more than 20 h in a solution with the dye and salt concentration for which the membrane was to be used. The final step was required since the dye's solubility in the dialysis membrane is a function of salt concentration. Fresh sections of dialysis membrane were used for each test, the tests were repeated five times each using solutions with no salt, 0.01 M NaCl and 1 M NaCl. Absorbance measurements were made on a Beckman DB-GT grating spectrophotometer.

The dye, diO-C_1-3-X, was supplied by Nippon Kankoh-Shikiso Kenkyusho, Ltd., Okayama, Japan; diO-C_5-3-I was a gift from Professor Alan Waggoner, Amherst College, Amherst, MA, U.S.A. These dyes were used without purification. The commercial dye contained minor impurities which did not contribute to the optical absorption of aqueous dye solutions.

High field pulse measurements [20,21] were made by applying 'square wave' voltage pulses across the membrane through a 10^5 Ω shunt resistor and calomel electrodes (≈2 kΩ series resistance).

Results

The photo-voltage amplitude, risetime and falltime depended upon both the membrane lipid and aqueous solution salt concentration. Typical waveforms are illustrated in Fig. 2. The amplitude is defined as the maximum voltage excursion from the preillumination value. The risetime is the time required after the flash onset for the voltage to reach 80% of the amplitude. The falltime is the time required after the flash for the voltage to fall back to 50% of the amplitude.

Imposed transmembrane voltages vary the photo-voltage amplitudes and risetimes for membranes which contain oxidized cholesterol [14]. Low effective membrane resistance values, which may result from oxidized lipids, antibiotics, or shunt resistors of less than 10^8 Ω, produce short membrane RC time constants which modify the waveforms. Short resistance capacitance (RC) time constants obscure the falltimes, and may result in biphasic waveforms [14]. A 10^9 Ω shunt resistor was used for all traces shown in Fig. 2, and resulted in RC time constants of about 3 s for membranes not containing oxidized lipids. With no shunt resistor or oxidized lipids in the membrane, the RC time constants were often 40 s. The waveforms were independent of the shunt resistance value, providing that the falltime was much less than the membrane RC time.

Membranes prepared from oxidized cholesterol and other membranes prepared from air-oxidized phosphatidylcholine also exhibited variations in their photo-voltage waveforms with imposed transmembrane voltage. Membranes which did not contain oxidized lipids did not show these variations (from +60 to −60 mV). Thus the lipid oxidation products are responsible for

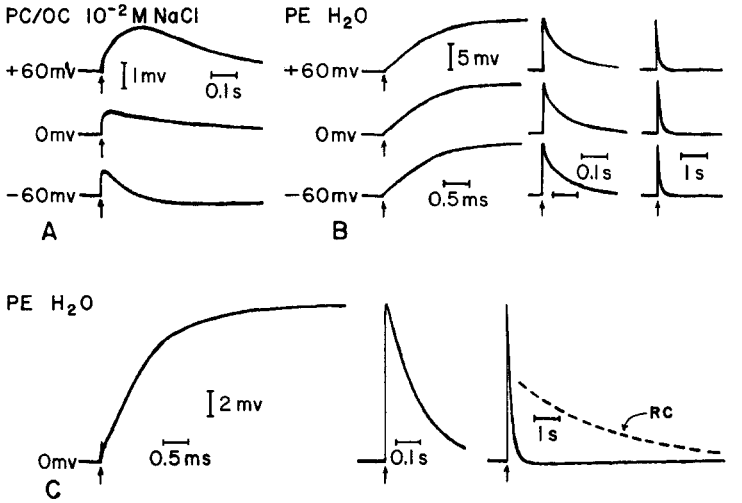

Fig. 2. Typical photo-voltage waveforms resulting from the flash illumination of bilayer membranes with diO-C_1-3-Cl at 10 μM in the positive electrode solution. (A) The waveforms for mixed phosphatidylcholine (PC), oxidized cholesterol (OC) membranes in 10^{-2} M NaCl solutions. The three traces shown were obtained with the transmembrane voltage adjusted to +60, 0, and −60 mV, respectively, by applying a current through a 10^9 Ω shunt resistor. Vertical arrows mark the time of the flash. (B and C) Successive traces obtained at three different oscilloscope sweep speeds for phosphatidylethanolamine (PE) membranes in distilled water solutions. The dashed line in C shows the 'RC' discharge of the membrane-10^9 Ω shunt resistor circuit after an applied current was suddenly switched to zero.

the transmembrane voltage-induced waveform variations.

The photo-voltage amplitudes, risetimes, and falltimes observed with various NaCl concentrations are given in Fig. 3. These results were reproduced for each lipid and salt concentration with NaBr, with the mean values falling within the range of values obtained for NaCl. Variations in the photo-voltage amplitude with dye concentration are given in Fig. 4.

These experiments were normally conducted with the same salt concentration on both sides of the membrane. However, experiments with different NaCl concentrations in the two aqueous solutions illustrated that the salt concentration on the dye side of the membrane strongly influenced the photo-voltages, while the salt on the other side had a smaller effect.

The results from the dialysis membrane experiments showed that solution passing through the dialysis membrane contained 56 ± 3% less dye when lipid was present than it did when no lipid was present, with no salt present. This quantity was 51 ± 9 and 28 ± 8% when the NaCl concentrations were 0.01 and 1 M, respectively.

The electric current transients resulting from voltage pulses are illustrated in Fig. 5. The current transients resulting from glycerol monoolein membranes in the presence of diO-C_1-3-Cl and diO-C_5-3-I were similar to the results with phosphatidylcholine except the membranes did not survive voltage pulses above 250 mV. These current traces could be compared for somewhat higher voltage values (to ≈350 mV), however, by reforming the membranes after each voltage pulse.

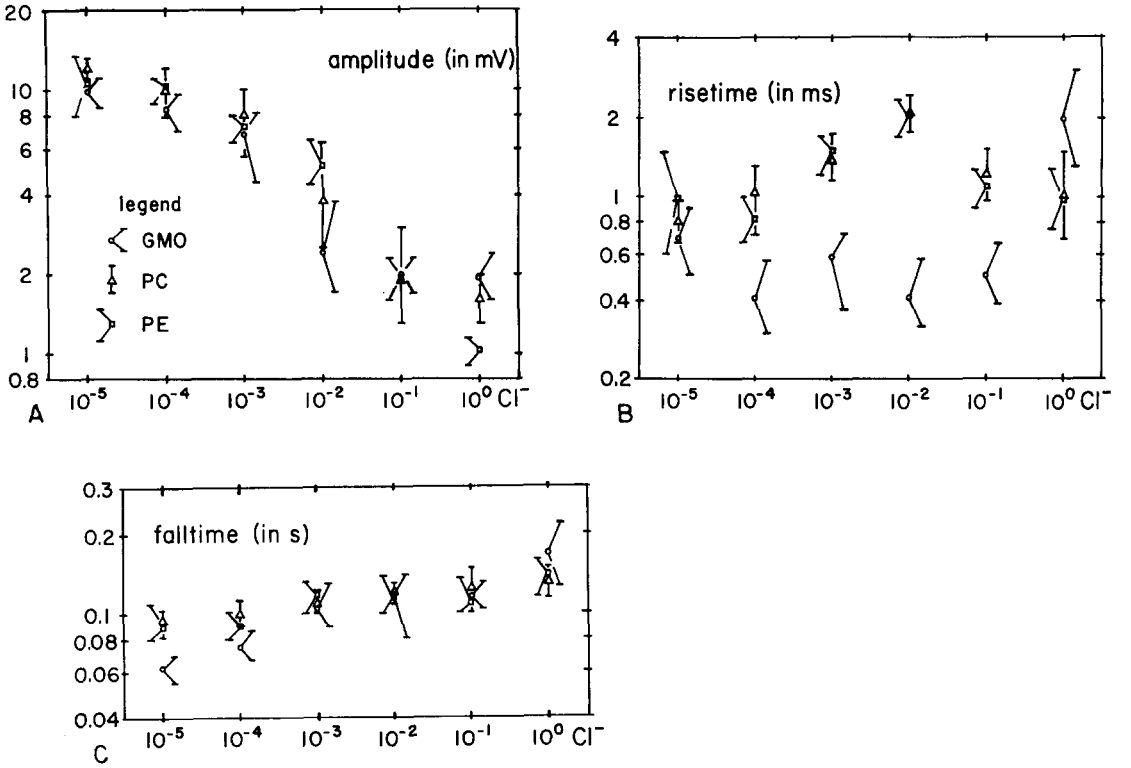

Fig. 3. Log-log plots of the mean values for the photo-voltage amplitudes (A), risetimes (B), and falltimes (C) versus chloride concentration in solution for glycerol monoolein (GMO), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) using 10 μM diO-C_1-3-Cl in the positive electrode solution. The tote marks give the range over which responses were observed. For clarity the tote marks are offset to the left for phosphatidylethanolamine and to the right for glycerol monoolein.

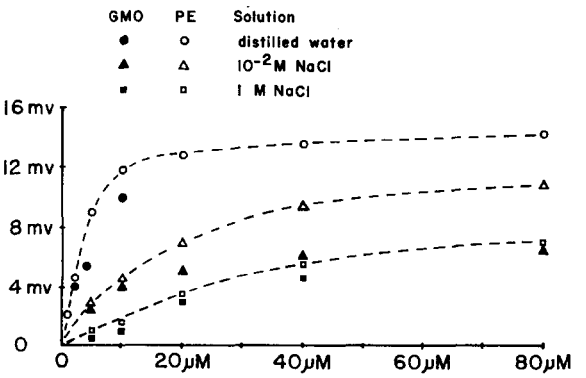

Fig. 4. The mean photo-voltage amplitudes versus diO-C_1-3-Cl concentration for glycerol monoolein (GMO) and phosphatidylethanolamine (PE) membranes in distilled water, 10^{-2} and 1 M NaCl solutions. The dye was added to the positive electrode solution only.

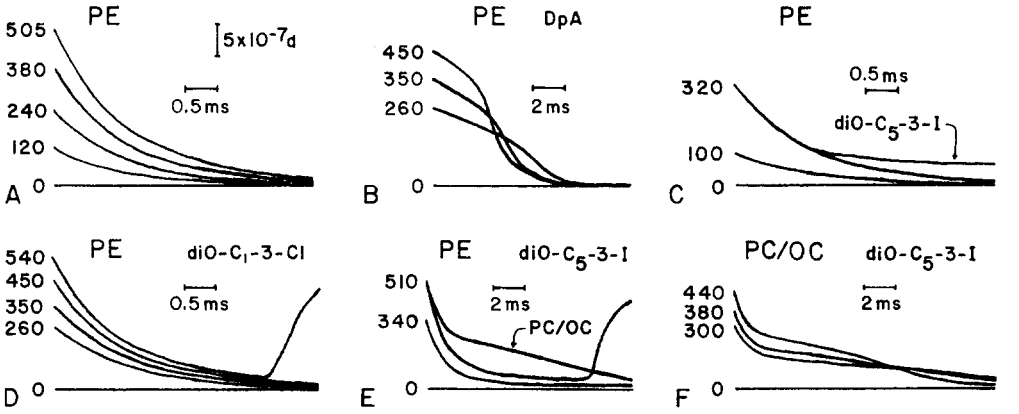

Fig. 5. The current transients resulting from 'square wave' voltage pulses being applied to the positive electrode solution, where the voltage pulse amplitude in mV is given immediately to the left of each current trace. In B, the dipicrylamine (DpA) was 1 μM in solutions on both sides of the membrane. In C, dye was added to the positive electrode solution after the 320 mV trace was recorded, and after 5 min the trace was repeated. All dye concentrations are 10 μM in the positive electrode solution only. In E, after the phosphatidylethanolamine (PE) membrane ruptured on the 510 mV trace, a mixed phosphatidylcholine (PC), oxidized cholesterol (OC) membrane was formed in the cell, allowed to go black, and the trace repeated. The solutions contained 10^{-2} M NaCl in A, B, and C, and 10^{-3} M NaCl in D, E, and F.

Discussion

The physical model of the membrane-dye system and its electric circuit equivalent are shown in Fig. 6. The membrane is divided into segments of equal area with each segment containing one sorbed dye molecule which will move upon illumination. An imaginary cylinder is cut through the membrane at the dye sorption site. The part of the cylinder on the dye side of the membrane forms the circuit elements C'_d and R'_d, while the other side forms C'_0 and R'_0. The portion of the membrane segment outside the cylinder forms C'_m and R'_m.

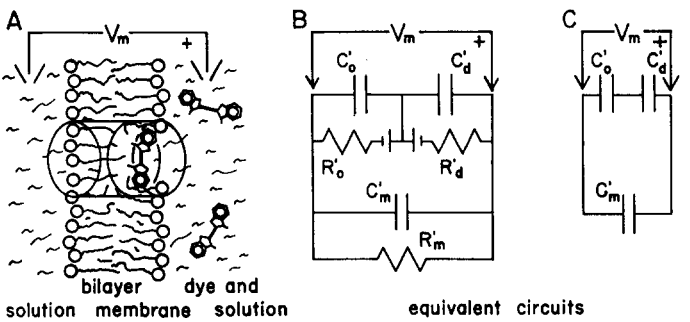

Fig. 6. The physical model (A) and equivalent circuit model (B and C) of a segment of the bilayer membrane with sorbed dye. The models assume that upon illumination the charged sorbed dye molecules move within the portion of the membrane identified as the capacitor C'_d, as described in the text. The circuit in C is valid for a time following the flash illumination that is short compared to $R'_0\,C'_0$, $R'_d\,C'_d$, and $R'_m\,C'_m$.

Each segment is equal in area, and n segments make up the total membrane. Upon flash illumination, a dye molecule moves across the capacitor C'_d to create an electric displacement, which creates the photo-voltage. The equivalent circuit model shown in Fig. 6C is valid for a time following the flash that is less than the smallest of the products $R'_d C'_d$, $R'_0 C'_0$ and $R'_m C'_m$. See Huebner [14] for a more detailed analysis of this equivalent circuit model.

Sorbed dye molecules occupy a small percentage of the membrane surface area, so with a small error, $C_m = n C'_m$. Assuming that each charge that moves has a value of e, then the photo-voltage amplitude can be estimated from Eqn. 6 of Huebner [14] as,

$$V_{max} = \frac{ne}{C_m n C'_d (1/C_m + 1/nC'_d + 1/nC'_0)} \tag{1}$$

where n is the number of dye molecules which move. Implicit in the model is the assumption that C'_0 and C'_d have the same cross sectional area, and their thicknesses sum to the membrane thickness, l. It is further assumed here that the dielectric constants of the materials which make up C'_0, C'_d and C_m are identical. Then by choosing C'_d to be xl thick, so that C'_0 is $(1-x)l$ thick, Eqn. 1 reduces to

$$V_{max} = \frac{xne}{C_m(1 + xnC'_d/C_m)} \simeq \frac{xne}{C_m} \tag{2}$$

The approximation follows since the surface of the membrane occupied by dye is small (i.e. $x\, n\, C'_d << C_m$). This equation indicates that the voltage increases linearly with dye displacement, so the photo-voltage risetime provides a measurement of the rate of dye movement.

This theory predicts variations in the amplitude will occur with variations in three factors, C_m, x, and n. White [22] has shown that C_m increases by approx. 28% for glycerol monoolein membranes when the salt concentration is increased from 10^{-4} to 1 M NaCl. This is explained by variations in the spatial distribution of polarization charge [23,24]. At higher salt concentrations, when the Debye length is reduced, the polarization charge is closer to the hydrocarbon membrane core, reducing l, the effective thickness of C_m. If dye molecules are sorbed at the edge of the hydrocarbon core, then increasing the salt concentration should reduce x, as in the limit of high salt concentration when C_m approaches the geometric capacitance of the hydrocarbon core, x goes to zero. Thus x is expected to be reduced by increased salt concentrations. The dialysis membrane experiments indicate that n is also reduced with increased salt concentration.

It should be noted that bilayer membranes and lipid suspensions may not give an accurate estimate of the amount of sorbed dye. Spherical micell surfaces may sorb dye differently than planar membrane surfaces. The bilayer membranes contained decane which may alter dye sorption, while the micells did not. In addition the amount of lipid present in the micell suspensions had to be sufficient to alter the dye concentrations in solution, while in the membrane case the dye concentration remained at 10 μM.

The above and other uncertainties prevent accurate calculations of n and x.

The other uncertainties relate to the fact that C_m varies with sorbed surface charge [23,24], and that the percentage of sorbed dye which moves upon illumination may vary with salt concentration of the aqueous solutions. Nevertheless, a range of values possible for n and x may be estimated from the data presented here. Rearranging Eqn. 2 yields

$$n \simeq \frac{C_m V_{max}}{xe} \tag{3}$$

Assuming $C_m = 4$ nF/mm^2, $l = 60$ Å, $x = 0.2$, and $V_{max} = 12$ mV, then $n = 1.5 \cdot 10^9$/mm^2. This corresponds to one dye moving 12 Å for every ≈1700 lipid molecules on the dye side of the membrane. Assuming C_m and $1/x$ increase by 4% per order of magnitude increase in anion concentration, Eqn. 3 predicts that one dye moves for each ≈4000 and ≈9000 lipid molecules, respectively, in 0.01 and 1 M NaCl. These numbers may be compared to the dialysis membrane results which indicate that one dye molecule sorbs for every 470, 520 and 930 lipid molecules in distilled water (10^{-5} M anion concentration, because of the dye), 0.01 and 1 M NaCl, respectively. The percentage of sorbed dye molecules moving then is 28, 13 and 10%, respectively.

It is also of interest to assume that n has the value determined from the dialysis membrane experiments, and that all n dye molecules sorbed in the membrane move upon flash illumination. Eqn. 3 may alternatively be written as

$$x \simeq \frac{C_m V_{max}}{ne} \tag{4}$$

Using the C_m values from above, then x equals 5.6, 2.3 and 1.7% of the membrane thickness for distilled water, 0.01 and 1 M NaCl, respectively. These values must be taken as a lower limit for two reasons. (i) Reductions in illumination intensity result in linear reductions in amplitude. It is inferred from that that increasing the light intensity would increase the amplitude, additionally increasing n, thus the n values used to calculate x were too large, and the x obtained above is too small. (ii) These calculations assume the quantum efficiency of photo-isomerization is unity, which is unlikely, again indicating x is too small.

It may have been expected from electrostatic considerations that more dye would sorb to the lipid surface at increased salt concentrations, when electrostatic screening is more effective. An increased salt penetration of the membrane surface layer seems likely to result in different inter-molecular organizations. Salt single ions, double ions, as well as triple ions (Na_2Cl^+), which may form in the membrane surface at higher NaCl concentrations [25,26], may be involved. Membranes formed using different lipids must, of course, have different surface structures. The risetimes are seen (Fig. 3B) to vary with lipid head group structure, with a faster risetime resulting for glycerol monoolein. This is consistent with the view that risetime variations with increased salt concentration result from a modified membrane surface structure.

The specific capacitance of glycerol monoolein membranes indicates that in 1 M salt solutions a modified hydrocarbon core structure results [22], perhaps from salt ions penetrating the core's outer regions. An abnormally long rise-

time would be expected from dye penetrating the hydrocarbon core. The long risetimes obtained for glycerol monoolein membranes at 1 M NaCl and NaBr provides support for this view.

Two other recent studies provide results that are also consistent with the interpretation given here, namely that changing the aqueous solution salt concentration modifies the membrane structure, and modified membrane structures result in modified photo-voltage waveforms. These studies show that indole-3-acetic acid distribution in bilayer membranes depends upon the aqueous solution KCl concentration [27,28], and that thermally induced phase transitions in phosphatidylcholine suspensions depend upon the type and concentration of ions in solution [29].

The transmembrane currents which flow following the application of a voltage 'square wave' in the absence of dyes or lipophilic anions result from charging C_m. These current transients increase linearly with the voltage pulse amplitude, and decrease exponentially in time following the onset of the voltage pulse, as illustrated in Fig. 5A. The presence of dipicrylamine adds an additional current (see Fig. 5B), which results from sorbed dipicrylamine anions being driven across the membrane [20]. This additional current transports only sorbed ions. Higher amplitude voltage pulses reduce the transport time; consequently the current transients 'cross over' on applied voltage pulses of increasing amplitude [20].

The traces shown in Fig. 5 illustrate that diO-C_1-3-Cl cannot be driven across phosphatidylcholine membranes with 500 mV. That seems surprising, since diO-C_1-3-Cl and diO-C_5-3-I appear more lipophilic than dipicrylamine, and are known to have delocalized charge [30]. A type of 'crossing over' was observed with diO-C_5-3-I in membranes containing oxidized lipids but the current traces did not return to the baseline (see Fig. 5F). Crossing over was not observed with diO-C_5-3-I in membranes which did not contain oxidized lipids, although an enhanced current was observed (see Fig. 5C). Crossing over was not observed with diO-C_1-3-Cl in membranes containing oxidized lipids.

The presence of oxidized lipids in bilayer membranes apparently causes relatively polar regions to form, which allow dye cations to be pushed into the membrane with positive voltages, which produces the risetime and amplitude variations previously described [14]. These polar regions apparently allow dye to cross the membrane more readily, lowering membrane resistance, and facilitating dye transport with voltage pulses. The transport of lipids across vesicle bilayers by lipid oxidization products has also been recently reported [31].

The photo-voltages described here demonstrate the existence of significant populations of relatively long lived photo-modified states of cyanine dye molecules in membranes. The effects of these states on experiments using cyanine dyes for photometrically monitoring transmembrane potentials are uncertain. It would be helpful to know the lifetime, absorbance and fluorescence spectra and intensity of the photo-modified dye states. The absorbance changes and lifetime for a similar dye, diS-C_2-3-I, undergoing photo-isomerization in ethanol are described by McCartin [32]. Other references and a brief discussion are included in Ref. 14. The percentage of dye in the photo-modified state in membranes would decrease with reductions in light intensity from the $\approx 5 \cdot 10^9$ lux

used here, but it would be increased with the duration of illumination from the 7 μs used here. Although other dyes have yet to be studied in sufficient detail with these methods to permit an estimate of the percentage of dye which would be in the photo-modified state, experiments with other carbocyanine dyes which produce photo-voltages [13,14] verify that these effects are general to this class of dyes. The present results clearly suggest that results with other charged dyes should be expected to vary with membrane lipid composition and aqueous solution salt concentrations.

Acknowledgements

We thank Ray Bowman for advice on preparing the dialysis membranes, Erich Brumbaugh for advice on the manuscript, Steven White for advice on lipid purity and membrane stability, and Jeanne Huebner for typing the manuscript. This work is supported by the National Institutes of Health through grant GM 23250.

References

1 Laris, P.C., Pershadsingh, H.A. and Johnstone, R.M. (1976) Biochim. Biophys. Acta 436, 475—488
2 Burckhardt, G. (1977) Biochim. Biophys. Acta 468, 227—237
3 Kashket, E.R. and Wilson, T.H. (1974) Biochem. Biophys. Res. Commun. 59, 879—886
4 Laris, P.C. and Pershadsingh, H.A. (1974) Biochem. Biophys. Res. Commun. 57, 620—626
5 Brewer, G.J. (1976) Biochemistry 15, 1387—1392
6 Salama, G. and Morad, M. (1976) Science 191, 485—487
7 Waggoner, A.S. (1976) J. Membr. Biol. 27, 317—334
8 Laris, P.C., Bahr, D.P. and Chaffee, R.R.J. (1975) Biochim. Biophys. Acta 376, 415—425
9 Hoffman, J.F. and Laris, P.C. (1974) J. Physiol. London 239, 519—552
10 Sims, P.J., Waggoner, A.S., Wang, C.H. and Hoggman, J.F. (1974) Biochemistry 13, 3315—3330
11 Ullrich, H.M. and Kuhn, H. (1969) Z. Naturforsch. 24b, 1342
12 Ullrich, H.M. and Kuhn, H. (1972) Biochim. Biophys. Acta 266, 584—596
13 Huebner, J.S. (1975) Biochim. Biophys. Acta 406, 178—186
14 Huebner, J.S. (1978) J. Membr. Biol. 39, 97—132
15 Infelta, P.D., Gratzel, M. and Thomas, J.K. (1974) J. Phys. Chem. 78, 190—195
16 Thomas, J.K. (1977) Acc. Chem. Res. 10, 133—138
17 Tien, H.T. and Huebner, J.S. (1973) J. Membr. Biol. 11, 57—74
18 Fieser, L.J. (1963) in Organic Synthesis (Rabjoh, N., ed.), pp. 195—201, John Wiley and Sons, Inc., New York
19 Tien, H.T., Carbone, S. and Dawidowica, E.A. (1966) Nature 212, 718—719
20 Bruner, L.J. (1975) J. Membr. Biol. 22, 125—141
21 Andersen, O.S. and Fuchs, M. (1975) Biophys. J. 15, 795—830
22 White, S.H. (1973) Biochim. Biophys. Acta 323, 343—350
23 Läuger, P., Lesslauer, W., Marti, E. and Richter, J. (1967) Biochim. Biophys. Acta 135, 20—32
24 Everitt, C.T. and Haydon, D.A. (1968) J. Theor. Biol. 18, 371—379
25 Robinson, R.A. and Stokes, R.H. (1959) Electrolyte Solutions, 2nd edn., p. 402, Butterworth, London
26 Harned, H.S. and Owen, B.S. (1958) The Physical Chemistry of Electrolytic Solutions, 3rd edn., pp. 74 and 300, Reinhold, Amsterdam
27 Zimmermann, U., Steudle, E. and Lelkes, P.I. (1976) Plant Physiol. 58, 608—613
28 Zimmermann, U., Ashcroft, R.G., Coster, H.G.L. and Smith, J.R. (1977) Biochim. Biophys. Acta 469, 23—32
29 Chapman, D., Peel, W.E., Kingston, B. and Lilley, T.H. (1977) Biochim. Biophys. Acta 464, 260—275
30 Brooker, L.G.S. (1966) in The Theory of the Photographic Process (James, T.H., ed.), 3rd edn., pp. 198—232, Macmillan Co., New York
31 Shaw, J.M. and Thompson, T.E. (1978) Biophys. J. 21, 122a
32 McCartin, P.J. (1965) J. Chem. Phys. 48, 2980—2981