BBA 46238

ELECTRICAL TRANSIENTS OF A CHLOROPLAST BIMOLECULAR LIPID MEMBRANE ELICITED BY LIGHT FLASHES

J. S. HUEBNER AND H. TI TIEN

BLM Research Laboratory, Department of Biophysics, Michigan State University, East Lansing, Mich. 48823 (U.S.A.)

(Received July 13th, 1971)

SUMMARY

Using flash excitation, the photo-responses of bilayer lipid membranes made of chloroplast lamella extracts have been studied as a function of: (i) a chemical ($FeCl_3$) gradient, (ii) a pH gradient, and (iii) an electrical potential gradient. Combinations of these gradients have also been studied. Three distinct photo-activated charge transport processes are identified. The fastest component is seen to alter the transmembrane electric potential within 1 μsec (the time accuracy limit of these measurements) of the initial illumination. Two slower components continue to actively alter the transmembrane potential for approximately 20 msec and 1 sec, respectively, after the illumination period. The results are interpreted in terms of the movement of photo-generated electronic carriers and protons.

INTRODUCTION

Recent work has shown that bimolecular lipid membranes containing pigments such as chlorophylls or retinals exhibit interesting photoelectric effects[1-3]. The photo-responses of these pigmented bimolecular lipid membranes had been found to be complex, varying from simple monophasic to biphasic waveforms when the experimental parameters were altered. In preliminary experiments using an electronic stroboscope as a light source, evidence indicated that the photo-response of retinal bimolecular lipid membranes could be exceedingly rapid[4]. In this paper we report the results of an investigation of light-induced electrical transients in bimolecular lipid membranes formed from chloroplast lamella extracts. We have found that the appearance of photo-electromotive force across the bimolecular lipid membrane requires less than 1 μsec, and the waveforms of the photo-response can be altered by (i) a chemical ($FeCl_3$) gradient, ΔC, (ii) a hydrogen ion gradient, ΔpH, and (iii) an electric potential gradient, ΔV. The effects of the combinations of these gradients across the bimolecular lipid membrane have also been examined.

MATERIALS AND METHODS

The laboratory distilled water was re-distilled in an all-glass apparatus before use. The chemicals and solvents were reagent grade and were used as obtained.

Fresh spinach leaves obtained from a local market were washed, and had the stocks and midstems removed prior to use.

The extraction procedure was performed, in brief, by: (i) chopping the leaf parts in a Kenmore blender (Sears, Roebuck and Co., Chicago, Ill., 60607, U.S.A.) in 0.5 M sucrose–0.05 M $KHCO_3$ solution at pH 7.5; (ii) this mixture was filtered; (iii) rinsed by centrifugal precipitation and resuspension in additional buffered sucrose solution; (iv) then after centrifugal precipitation, the precipitate was resuspended in distilled water; (v) after centrifugally precipitating again, the precipitate was mixed with light petroleum–methanol (2:1, v/v) in the blender; (vi) after centrifugal separation, the ether soluable phase was dried in a flash evaporator (Buchler Instruments, Fort Lee, N.J. 07024, U.S.A.); and (vii) the residue dissolved in *n*-butanol–dodecane (1:1, v/v) was ready for bimolecular lipid membrane formation.

The bimolecular lipid membranes were formed on a 2-mm diameter hole in a Teflon cup in the usual arrangement by depositing approx. 2 μl of the lipid solution from a microsyringe (100 μl) with a repeating dispenser attachment (Model PB-600, Hamilton Co., Inc., Whittier, Calif. 90605, U.S.A.). The membranes were observed to thin to the black state in symmetrical solutions using dim green light. The chemical or pH gradients could then be established by removing a small volume of one solution and replacing it with an equal volume of concentrated $FeCl_3$, HCl or KOH solution. This procedure was accomplished by using a micropipette (Sampler, Oxford Laboratories, San Mateo, Calif. 94401, U.S.A.) and was followed by magnetic stirring for a 15-min period in the dark.

The transmembrane potential was measured by calomel electrodes (39270, Beckman Instr. Inc., Fullerton, Calif. 92634, U.S.A.) in each solution connected to a high input impedance amplifier (MPA-2, Transidyne General Corp., Ann Arbor, Mich. 48103, U.S.A.). The amplifier output was displayed on an oscilloscope screen and photographed. The electric potential gradients were applied through calomel electrodes using a battery, potentiometer, and 10^8–10^{10}-Ω resistors (Hi-meg Resistors, Victoreen Instr. Co., Cleveland, Ohio 44104, U.S.A.); the output resistance of voltage source was kept at least an order of magnitude larger than the 10^7–10^9 Ω dark bimolecular lipid membrane resistance. The light source was a xenon flashtube (Stroboslave 1539-A, General Radio Co., West Concord, Mass. 01781, U.S.A.); the stroboscopic flashes and oscilloscope sweeps were triggered by a transistor timing circuit. The flash intensity of the stroboscope had a peak value of 10^9 lux at the bimolecular lipid membranes, the 1/3 intensity points were separated in time by about 3 μsec. This intensity could be reduced by inserting grey neutral density filters (Carl Zeiss, Inc., West Germany) in the light path. All measurements were made at room temperature (approx. 23°).

RESULTS

The flash photo-voltage waveforms resulting from each of the three different asymmetrical conditions (ΔC, ΔpH and ΔV) are shown in Fig. 1. The photo-response of each bimolecular lipid membrane was reproducible and predictable; however, changes in the amplitude often occurred with repeated flashes, as will be described below. The ΔC photo-response consisted of three distinct components, which can be seen clearly at progressively slower sweep rates (Fig. 1a). The three components are

designated as follows: (1) Component A, a rapid change in the membrane voltage which occurs principally during the flash excitation, (2) Component B, a slower change in voltage, which reaches a peak value about 20 msec after the flash excitation, and (3) Component D, the decay of the membrane voltage in the dark. The magnitude of the photo-electromotive force due to both Components A and B was reduced when: (i) the light intensity was reduced, (ii) the pH of the bathing solution was either lower than 4 or higher than 6, and (iii) the membrane was excited repeatedly with flashes. The polarity of the ΔC induced photo-response (i.e. Components A and B) were always in the direction to make the solution containing $FeCl_3$ negative.

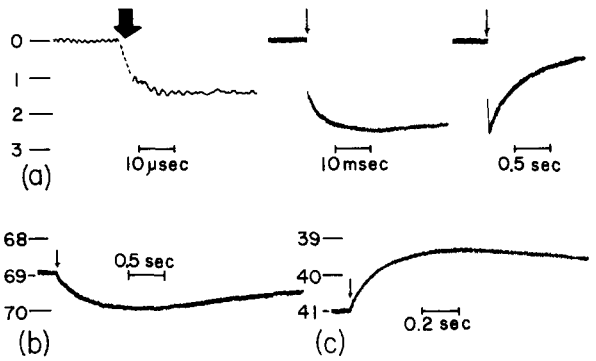

Fig. 1. The photovoltage waveforms (in mV) resulting from a single flash excitation (see arrows) of the chloroplast bimolecular lipid membrane subjected to the three types of asymmetrical conditions. (a) The ΔC response in 0.1 M acetate buffer solutions at pH 5.5. $FeCl_3$ was added to one solution to 0.001 M strength after the membrane had become black. Successive traces were recorded at lower sweep speeds. (b) The ΔpH response in 0.1 M KCl solutions with pH valves of approx. 5.5 and 4.2. The pH gradient produced the 69-mV dark potential, with the low pH solution being negative. (c) The ΔV response in 0.1 M acetate buffer solutions at pH 5.0. An external voltage was applied to produce the 41-mV dark voltage.

Some typical ΔpH and ΔV photoresponses are shown in Figs. 1b and 1c, respectively, where the peak values are seen to occur at about 1.3 and 0.5 sec after the flash excitation. In these cases, the much slower change in the membrane voltage is designated as Component C, to differentiate it from Component B. The polarity of the ΔpH photo-response was such as to cause a further increase in the dark bimolecular lipid membrane polarization (*i.e.* to cause the potential of the low pH solution to become additionally negative) and was much larger in the unbuffered bathing solutions (0.1 M KCl). In contrast, the ΔV photo-response caused a decrease in the externally applied membrane potential. This photo-induced voltage decrease increased in magnitude nearly linearly with the magnitude of the applied voltage. The long duration of Component C in the ΔpH and ΔV photo-responses caused the membrane voltage to differ from the dark value for periods of several to many times the dark bimolecular lipid membrane discharge time.

Fig. 2 shows typical photo-voltage waveforms observed for bimolecular lipid membrane subjected to combinations of ΔC, ΔpH, and ΔV. In the case of the ΔC, ΔV combination, the electrical transients may be arranged to be biphasic, with Components A and B opposite in polarity to Component C (Fig. 2a). Similar biphasic responses were also observed with the ΔC, ΔpH combination. Since ΔC, ΔpH, and ΔV

may each be independently reversed with respect to each other, monophasic photo-responses were also obtainable from the $\Delta C,\Delta$pH and $\Delta C,\Delta V$ combinations (Fig. 2b). The presence of Fe^{3+} in either or both bathing solutions resulted in an enhancement of Component C. The 5- to 40-fold enhancement occured independent of the location of the Fe^{3+}, however, the photovoltage reached its peak value in 30–50 % less time if the direction of the photo-response for ΔC (*i.e.* Components A and B) and Component C were the same. This shifting of the photo-response peak to an earlier time is illustrated in Fig. 2b, and is larger than expected from a simple addition of the components

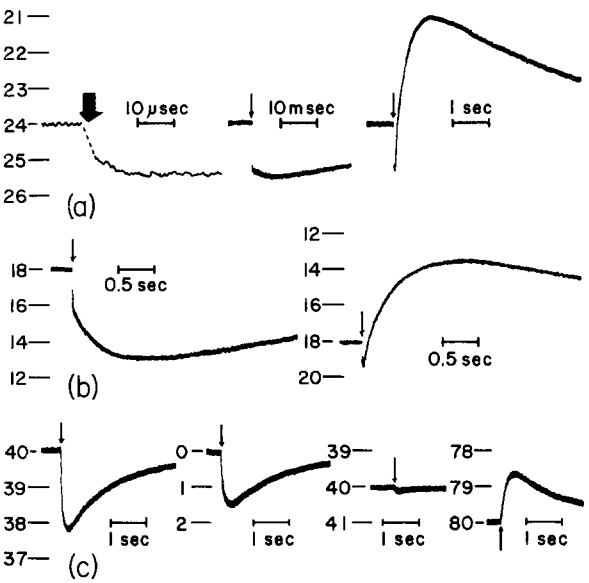

Fig. 2. The photovoltage waveforms (in mV) resulting from a single flash excitation (see arrows) of the chloroplast bimolecular lipid membrane subjected to combinations of the asymmetrical conditions. Aqueous solutions were all 0.1 M acetate buffer; pH values in (a) and (b) were 5.5. (a) The $\Delta C,\Delta V$ biphasic response showing successive traces at lower sweep speeds. The 24-mV dark voltage was produced by an externally applied voltage. (b) The $\Delta C,\Delta V$ response of another bimolecular lipid membrane illustrating the effect of reversing ΔV (from $+18$ to -18 mV). (c) The ΔpH,ΔV response with $FeCl_3$ added to both solutions to 0.001 M strength. The pH values were 4.6 and 5.2; responses are shown for four ΔV values, from left to right: 40, 0, -40, and -80 mV. The dark voltage with the external voltage source removed was about -40 mV. The photo-response prior to the $FeCl_3$ addition with the dark voltage held at 0 mV was about -0.3 mV.

In the absence of ΔC (*i.e.* with equal concentrations of $FeCl_3$ in both solutions or no $FeCl_3$ present in either solution) Components A and B were non-existent. The magnitude of the voltage change due to Component A could also be altered by the magnitude and polarity of ΔV. In one experiment, the positive magnitude of Component A was reduced by half with the application of an external voltage to produce a dark voltage of $+80$ mV, and enhanced by a factor of 2 when the dark voltage was -80 mV. Occasionally, the magnitude of Component A would vary with time as a voltage was applied. Component C from ΔV increased linearly as a function of the magnitude of the dark voltage, with a plot of the response magnitude versus dark voltage passing through the origin (*i.e.* Component C = 0 at $\Delta V = 0$ with no ΔpH). A linear plot was also obtained for the component C response from ΔV,ΔpH combination,

only the zero photo-response was shifted to a dark voltage value somewhat greater than the open circuit dark voltage which resulted from the pH gradient[3] (Fig. 2c). A biphasic photo-response could not be obtained with ΔV,ΔpH combinations.

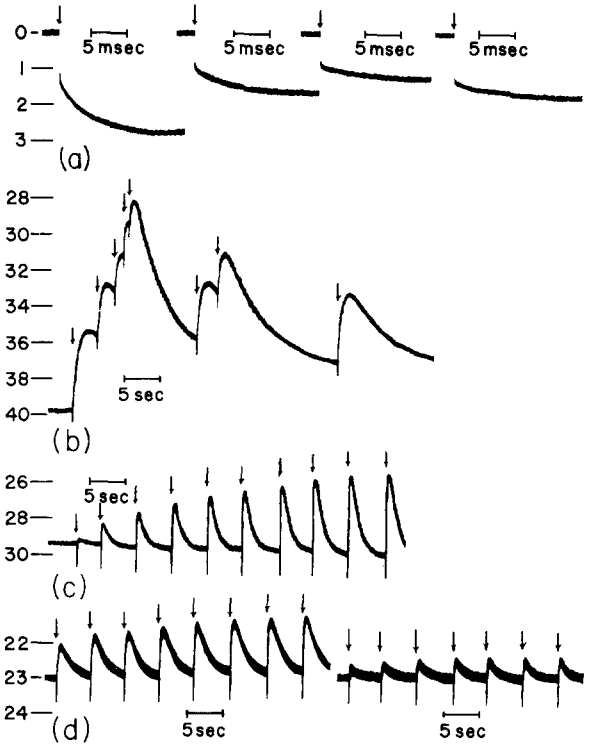

Fig. 3. The photovoltage waveforms (in mV) resulting from multiple flash excitation (see arrows) of chloroplast bimolecular lipid membranes, illustrating three types of behavior. Aqueous solutions were all 0.1 M acetate buffer; pH values were 5.5 in (a) and (b) and 6.0 in (c) and (d). (a) The reduction and recovery of the ΔC response. From left to right, the traces show the initial response, the response after 500 flashes at 20 flashes per sec and a 1-min dark period, the response after an additional 500 flashes and 1-min dark period, and the response after a 15-min dark period. (b) The voltage build-up from a ΔC,ΔV biphasic response. (c) The ΔC,ΔV response showing the Component C amplitude enhancement on subsequent flashes. (d) The ΔC,ΔV response of another bimolecular lipid membrane showing the Component C amplitude enhancement on subsequent flashes. The left trace shows the initial response, the right trace after a 15-min dark period and with a 10^8-Ω shunt resistor.

Three types of variations in the photovoltage waveforms were observed with multiple flash excitation. First, with an exception noted below, the photo-response amplitude was gradually reduced with each succeeding flash so that after 1000 flashes, typically 50–70% of the initial response remained. For the ΔC response, Component A would usually recover its initial amplitude if left in the dark for 5–15 min, while Component B would only partially recover. This process is illustrated in Fig. 3a. After 3000–6000 flashes, Component B was usually completely eliminated. When Component C was produced by ΔpH or ΔV only, a comparable amplitude reduction and recovery occurred. If component C was enhanced by $FeCl_3$, then Component C would often fail to recover completely. Second, if insufficient

time was allowed for the voltage to return to the dark value, a progressive voltage build-up took place, as is shown if Fig. 3b. The third way in which the photo-response varied with multiple flash excitation is illustrated in Figs. 3c and d. A time interval of about 5 sec between flashes was allowed to prevent the voltage build-up just described. The photo-response amplitude to succeeding flashes increased to a maximum in about 10–15 flashes. An increase in the Component C amplitude of over an order of magnitude was observed. Components A and B often did not vary, but sometimes increased slightly after 10 flashes. This sequence of events was often completely repeatable after a 10–15-min interval in the dark. This interesting effect was only observed with Fe^{3+} in acetate buffer solution near pH 6. The second trace in Fig. 3d shows the effect of reducing the dark bimolecular lipid membrane resistance by a factor of about 4 with the use of a 10^8-Ω shunt resistor. The shunt resistor is seen to reduce the voltage of Component C, but otherwise leaves the photo-response unchanged. These results show clearly that the time constants associated with Components A, B, and C are independent of the R–C time constant of the bimolecular lipid membranes in the dark (*i.e.* the time required to discharge the membrane capacitance through membrane and shunt resistance).

DISCUSSION

Since the voltage across a bimolecular lipid membrane is proportional to the electric charge stored on the membrane capacitance, the time derivative of the open circuit voltage will be proportional to the total transmembrane current, or the voltage waveform may be considered as the time integral of the total trans-membrane current. The photovoltage waveforms illustrated in this work provide direct evidence of three distinct photo-induced charge transport processes, which can account respectively for Components A, B, and C. Component A results from a photocurrent which, within the approximate microsecond time resolution of the present measurements, is sustained during the period of bimolecular lipid membrane illumination. Component B results from a smaller amplitude photocurrent, which is sustained for 20–30 msec after the flash excitation, and similar to that of Component A is reduced proportionally by the reduction of the flash intensity. Component C results from a still smaller but longer sustained photocurrent, which is some cases is sustained for several seconds. Owing to the long time duration of Component C, the discharge current (Component D) will introduce a nonlinear term, which reduces the photo-response amplitude of Component C, and prevents Component C amplitude from varying linearly with the flash intensity. From these data, a linear variation of Component C photocurrent magnitude with flash intensity would be anticipated. Component D is the decay of the membrane voltage to the pre-illumination value through the dark bimolecular lipid membrane resistance and in the absence of Component C, exhibits the characteristic R–C time of the bimolecular lipid membrane.

The observed electrical transients elicited by light flashes may be explained in terms of a model in which the chloroplast bimolecular lipid membrane is considered as an ultrathin liquid crystal of low conductance separating two highly conducting aqueous solutions. Charges resulting from the dissociation of excitons migrate under the influence of the chemical and electrical potentials. The electric fields present may be in the order of 10^5 V/cm. When efficient electron acceptors (Fe^{3+}) are present at the

solution/membrane interfacial region, the rapid formation of an electrical field across the bimolecular lipid membrane is interpreted as due to the ejection of photo-electrons from the membrane to the electron acceptors, which presumably originate from the excited chlorophylls[3]. The fast appearance of the electrical field across the membrane with no detectable latency in the μsec range (the limit of our instruments) strongly suggests the production and separation of electronic charge carriers (Figs. 1a and 2a). The location of the electron acceptor which determines the polarity of the photo-electromotive force being always negative with respect to the acceptor-free side, is in accord with this explanation.

The long time duration of the photo-activated charge transport after the flash excitation implies the existence of long-lived excitons (most likely triplet) which, upon dissociation, contribute to the charge transport across the bimolecular lipid membrane. If so, the externally applied field should exert a strong influence on these charges in the membrane. Since the mode of conduction in these membranes may be ionic as well as electronic[1,4], translocation of ionic charges such as H^+ across the membrane is also subject to the effect of applied fields. Thus, by a judicious combination of ΔC, ΔpH, and ΔV, a variety of photovoltage waveforms can be obtained, as indeed has been observed.

ACKNOWLEDGEMENT

These investigations were supported by a grant from the U.S. National Institutes of Health.

REFERENCES

1 H. T. TIEN, *Nature*, 219 (1968) 272.
2 H. T. TIEN AND N. KOBAMOTO, *Nature*, 224 (1969) 1107.
3 H. T. TIEN AND S. P. VERMA, *Nature*, 227 (1970) 1232.
4 N. KOBAMOTO AND H. T. TIEN, *Biochim. Biophys. Acta*, 241 (1971) 129.