Bioenergetics (1974), 6, 1-19

Characterization of Photovoltage Transients in a Chloroplast BLM

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Received 11 March 1974

Abstract

Flash-elicited electrical transients across a pigmented bilayer lipid membrane (BLM), formed from extracts of spinach chloroplast, have been further investigated under a variety of asymmetrical conditions. Flash excitation clearly delineates two voltage generative processes distinct on the basis of initial phase kinetics: (1) a concentration difference of $FeCl_3$ across the BLM gives rise to a microsecond photovoltage transient with polarity, action spectrum and risetime that suggest that photo-electrons from excited chlorophyll reduce Fe^{+3}; and (2) there exists a flash-elicited slower electrical transient in the presence of applied electrical field or pH difference. This Slow Component fits the following kinetic description: $V(t) = V_O\ [1 - \exp(-t/RC)]\ \exp(-\lambda t)$ and peaks at approximately 200 ms, depending upon the membrane RC time constant value, according to: $t_{peak} = 1n[(K + \lambda)/\lambda]/K$ sec, where $K = (RC)^{-1}$. The Slow Component exhibits initially RC kinetics, followed by first-order "Lambda" decay, slower than, and independent of, the BLM RC time constant value. Chlorophyll triplet excited state quenching agents such as ascorbic acid eliminate completely the Slow Component (voltage or pH difference-induced) leaving the Fast Component ($FeCl_3$-induced) unmodified. The long-lived nature of the Slow Component together with this result suggests that it represents photopopulation of the chlorophyll triplet excited state in the membrane.

Introduction

In photosynthesis, the primary event must involve energy transduction in an apparatus made principally of lipids, proteins and pigments. There is abundant evidence for such an apparatus comprising ultrathin mem-

branous structure (100 Å thick) which has been appropriately termed *thylakoid* membrane [1]. The molecular organization of the thylakoid membrane although still obscure at present, has been speculated by many investigators [2, 3]. However, physical chemical characterization of the thylakoid membrane, especially in the understanding of primary energy transduction, has been difficult owing to the complexity and minuteness of the thylakoid. In pursuing the problem of energy conversion in photosynthesis, we have employed the extensively studied artificially constituted bilayer lipid membrane or BLM system [4].

This work was undertaken to investigate the nature of the photoflash-stimulated voltage transient in chloroplast bilayer lipid membranes (Chl-BLM) under application of chemical and electrical differences across the membrane [5]. Kinetically, there are two basic photoresponses: a microsecond duration chemically-induced response (Fast Component) and a slower pH or voltage increment-induced transient (Slow Component) peaking typically 200 ms after illumination. In this communication, a mechanism is proposed to explain the kinetics of the Slow Component of the Chl-BLM induced by flash excitation. In addition, we report the phenomenon of photoeffect quenching in Chl-BLM by compounds which are known to quench the chlorophyll triplet state in vitro.

Materials and Methods

Preparation of Chloroplast Lipid Extracts for BLM Formation

Fresh spinach purchased from local markets was stripped of ribs and stalks and washed with distilled water, dried thoroughly, and then added slowly to a Waring blender at low speed with 300 ml of isotonic 0.5 M sucrose plus buffer at pH 7.5. When all the leaves had been added, high speed was used for 30 seconds; the homogenized mixture was filtered through 4 to 6 layers of cheesecloth. The filtrate was centrifuged in a Sorvall (Model SS-1) in eight 40 ml quantities at 8300 rpm for 5 minutes. The supernatant was discarded. The remaining crude chloroplast was resuspended in buffered sucrose solution at 25 ml per 2 test tubes, bringing the total stock to 4 tubes containing 25 ml each. Resuspensions were facilitated by a Vortex mixer. These tubes were again centrifuged at 8300 rpm for 5 minutes, the supernatant discarded and the residue washed and mixed in hypotonic 25 ml glass-distilled water per tube and allowed to stand for 5 minutes. These tubes were centrifuged at 9000 rpm for at least 10 minutes and the supernatant discarded, leaving a residue that was next extracted with 90 ml of 2:1 (V/V) petroleum ether/methanol solution in the blender at medium speed for one minute. Using two tubes this mixture was centrifuged at 6600 rpm for 10 minutes and the top layer pipetted off into a dry round-bottomed flask and evaporated to dryness in a flash-evaporator.

This residue was dissolved in 5 ml of 1:1 n-butanol:dodecane and placed in a clean covered storage vial.

Measurement of Membrane Photoelectric Properties

The experimental arrangement employed is illustrated in Fig. 1. Membranes were formed across a 1 mm circular aperture in a 10 ml teflon cup placed in a glass cell. This cell provided for minimum light scattering and membrane image distortion. Saturated KCl-bridging calomel electrodes (Backman model 39270) established external circuitry contact with the Chl-BLM bathing media. The electrodes were connected to an impedance buffering Philbrick FET operational amplifier (model 1021) with output displayed and recorded on a Tektronix R5031 dual beam storage oscilloscope screen. As indicated, the membrane was also shunted with variable large resistance and series variable applied voltage sources. Microsecond flash illumination was furnished by a General Radio Stroboslave xenon flash tube assembly (1539-A) featuring single and multiflash modes. A large Faraday cage constructed of a wooden frame, fiberboard and copper screening grounded to the outer chamber electrode enclosed the BLM support cell including electrodes and buffer amplifier, shielding them from spurious external illumination and electrical noise.

The Chl-BLM bathing medium was 0.1 M acetate titrated with potassium hydroxide to pH 5.1 in laboratory-distilled water redistilled in an all-glass still. Preliminary to membrane formation undesirable input currents detected by the buffer amplifier were offset via a trim adjustment to correct the output signal to zero when the electrodes were shorted. Electrode asymmetry potentials typically did not exceed 1 mV. The BLM was formed by sweeping across the aperture with the tip of a microsyringe after a two microliter extrusion using the repeating dispenser attachment.

Membranes formed in 0.1 M acetate buffer solution thinned to the black state in approximately ten minutes. This thinning was observed directly through use of a green filter and dim flash light triggered repeatedly by the timing circuit or more carefully monitored by comparing membrane RC transient times as thinning progressed, as capacitance is inversely related to thickness. Since BLM resistance was measured independently by the voltage diver technique, $R_m = R_s \cdot E_m/(E - E_m)$, C_m was calculated using R_m and R_s in parallel as the R of "RC" in the circuit given in Fig. 1. The Chl-BLM exhibited uniform stable resistive and capacitive properties which were measured and recorded since photovoltage features were determined largely by these characteristic electrical properties. Typical values of R_m and C_m were 10^8 ohms and 5000 pF (10^6 ohm-cm^2 and 0.5 μF/cm^2),

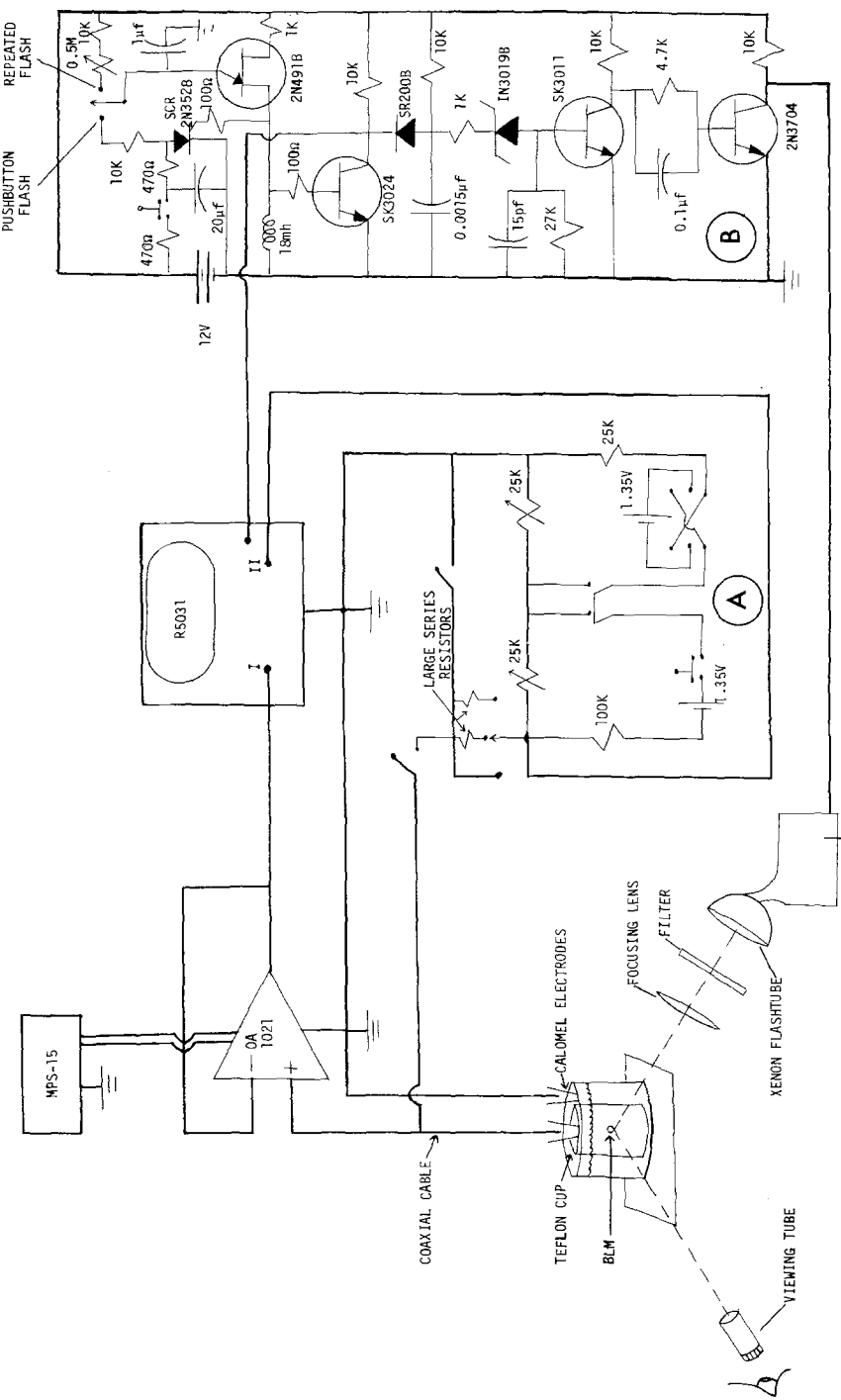


Figure 1. BLM flash excitation apparatus with dealy unit for resolving microsecond transients. (A) Series resistances and variable applied voltage source; (B) Oscilloscope and stroboscope trigger.

respectively. Subsequent to membrane stabilization, chemical or pH differences across the Chl-BLM were established by the addition of a small volume of concentrated HCl, KOH, $FeCl_3$, ascorbic acid, etc., to the inner chamber with the aid of an Oxford Sampler followed by careful magnetic stirring.

Results

The microsecond photoflash illumination of Chl-BLM technique has resolved single-flash voltage responses into clearly defined distinct components representative of kinetically independent membrane photoprocesses indistinguishable under continuous illumination circumstances [5]. Several combinations of applied voltage, chemical gradients, and pH differences were imposed across the membrane to induce observable effects. The voltage transients elicited were composed of four processes: a Fast Component with risetime in the microsecond range and induced by electron acceptors such as $FeCl_3$; a slower 100 to 300 ms Slow Component in the presence of a transmembrane pH or voltage difference; and two distinct voltage decay mechanisms, a simple RC decay corresponding exactly to the membrane RC transient in the dark and a slower decay transient, "lambda", a feature of pH difference and applied voltage-induced effects only. These four components essentially characterize all portions of waveforms elicited from Chl-BLM under flash excitation regardless of the nature of the transmembrane asymmetry. Also the great difference in component rates allows for clear delineation of both the fast and slow processes in response to a single flash, facilitating simultaneous evaluation of several components.

The Fast Component was detected only in response to certain chemical asymmetries across the BLM. For example, in the case of $FeCl_3$, a maximum photoresponse was elicited at a concentration of 2×10^{-3} M $FeCl_3$ in the inner ungrounded chamber. The $FeCl_3$ chamber potential was driven negative 3 to 5 mV upon flash illumination within 10 μs, (maximum instrumental response rate) and decayed according to the RC exponential transient characteristic of the BLM in the dark (Fig. 2a). These results were common to all Chl-BLM tested. Other electron acceptors such as ceric ion and methyl viologen induced responses of somewhat smaller magnitudes but with similar kinetics. Since $FeCl_3$ was one of the most effective sensitizers, it was employed in the majority of experiments investigating features of the Fast Component. As previously mentioned, the bathing medium was buffered with 0.1 M acetate and titrated to pH 5.1.

The Fast Component and accompanying RC decay appeared alone only in the total absence of applied voltage or pH difference; otherwise the Slow Component appeared to some extent. This presented moderate

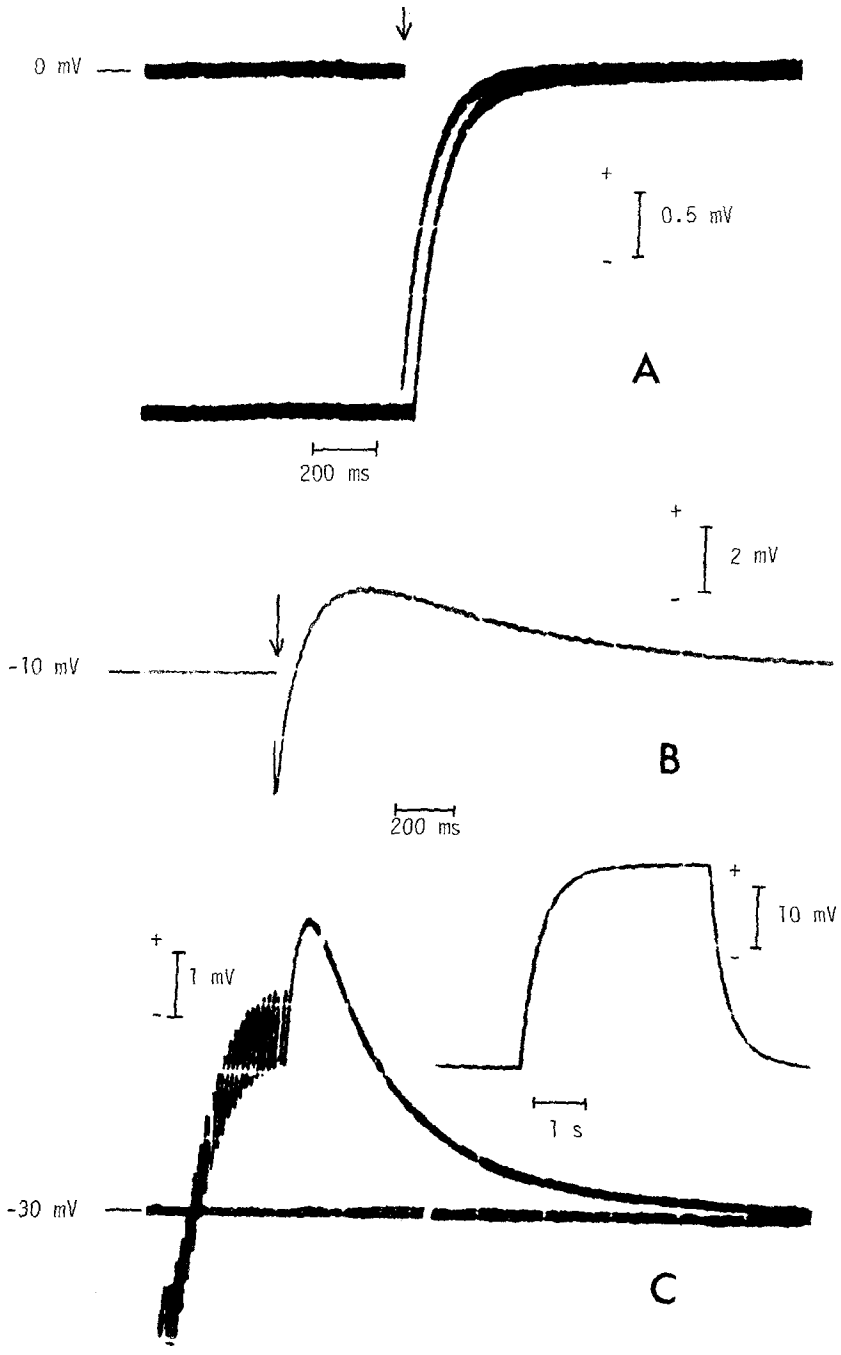
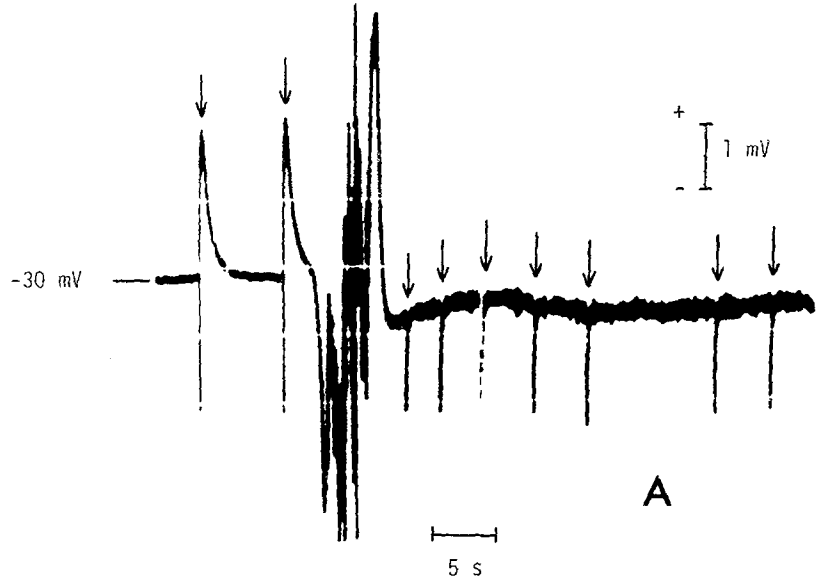

0 mV
0.5 mV
A
200 ms
2 mV
-10 mV
B
200 ms
10 mV
1 mV
1 s
-30 mV
C

Figure 2. The time course of chloroplast BLM photoresponses. (A) Response to microsecond xenon flash in the presence of 10^{-2} M $FeCl_3$ in the inner chamber compared to the dark chlorophyll-BLM RC transient. Arrow denotes flash. (B) Biphasic response in the presence of $FeCl_3$ and negative applied voltage (C) Chloroplast-BLM Slow Component decay kinetic (left) compared to membrane RC transient (right).

experimental difficulties; viz. $FeCl_3$, a Lewis acid, decreased the inner chamber pH on the order of 0.2 units, imposing a small pH asymmetry across the Chl-BLM and inducing slight Slow Component character in the response waveform. The Slow Component contribution, usually insignificant, could be additionally reduced in most cases via the external application of a slight reverse biasing voltage. A distinctive feature of the Fast Component was the fact that maximum possible applied voltages of 70 mV, corresponding to 100,000 V/cm field strength across the Chl-BLM, did not alter the polarity of the fast response; it continued to exhibit a polarity indicative of negative charge flow to the $FeCl_3$/Chl-BLM interface. The magnitude of the response was altered less than 15% under application of these intense fields, augmented when the field was of a polarity to assist negative charge flow to the $FeCl_3$/Chl-BLM interface and diminished when the field was oriented to retard current flow to this interface.

Upon flash excitation the Slow Component appeared in the presence of applied voltages or transmembrane pH differences. Its risetime was on the order of 0.5 sec and its polarity in the instance of applied voltage was such that the absolute magnitude of potential across the BLM in the dark prior to flash was reduced. Also, the flash response amplitude was directly proportional to the potential applied. An example of the addition of a positive Slow Component to the negative $FeCl_3$-induced Fast Component when a negative potential is applied is given in Fig. 2b. The magnitude of the Slow Component light response was increased by the addition of $FeCl_3$ in equal concentrations to both sides of the membrane. This concentration symmetry minimized fast response contributions to the photo-response, facilitating study of the Slow Component alone. The presence of $FeCl_3$, in addition to inducing the large fast photoresponse mentioned previously, enhanced the kinetically independent Slow Component approximately twenty-fold at 10^{-4} M concentrations. The pH-induced photoresponse exhibited kinetics similar to those of the applied voltage response but its polarity was always negative with respect to the high pH side of the membrane.

Whereas the Fast Component was discharged at a rate given by the Chl-BLM RC time, the Slow Component returned to the dark voltage value at a much slower rate. Fig. 2c compares an RC transient with a voltage-induced rapid repeated flash photoeffect decay process for the same membrane. This multiflash technique yielded a better-resolved


+
1 mV
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-30 mV
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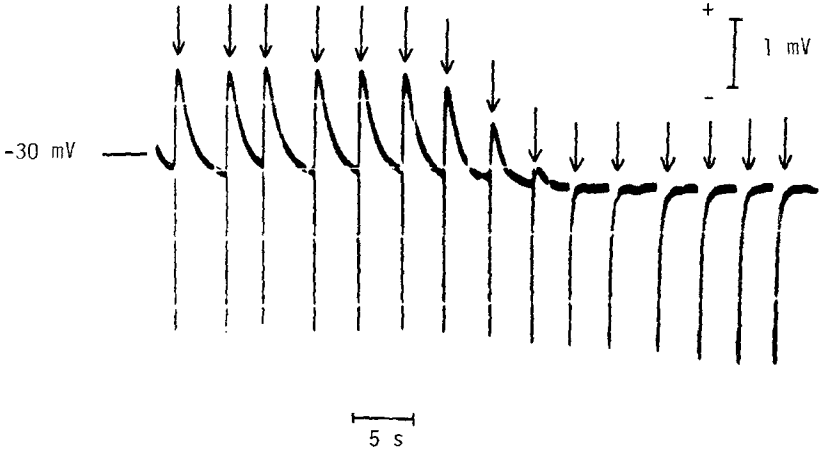
5 s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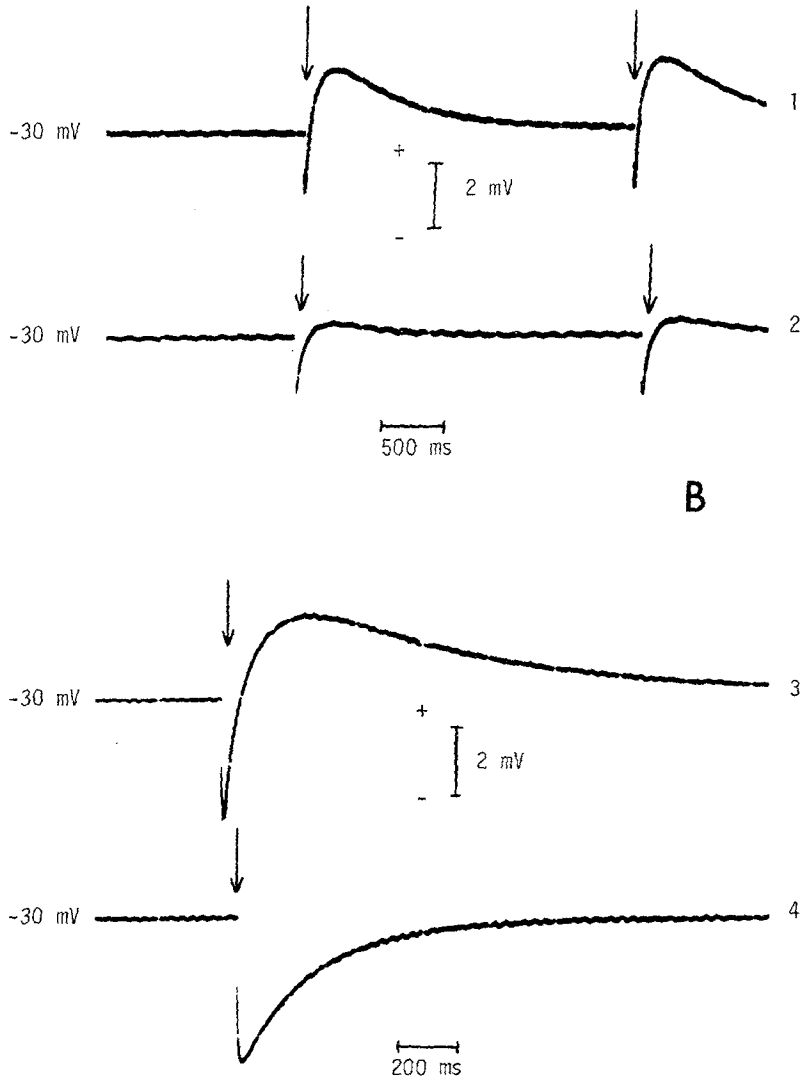
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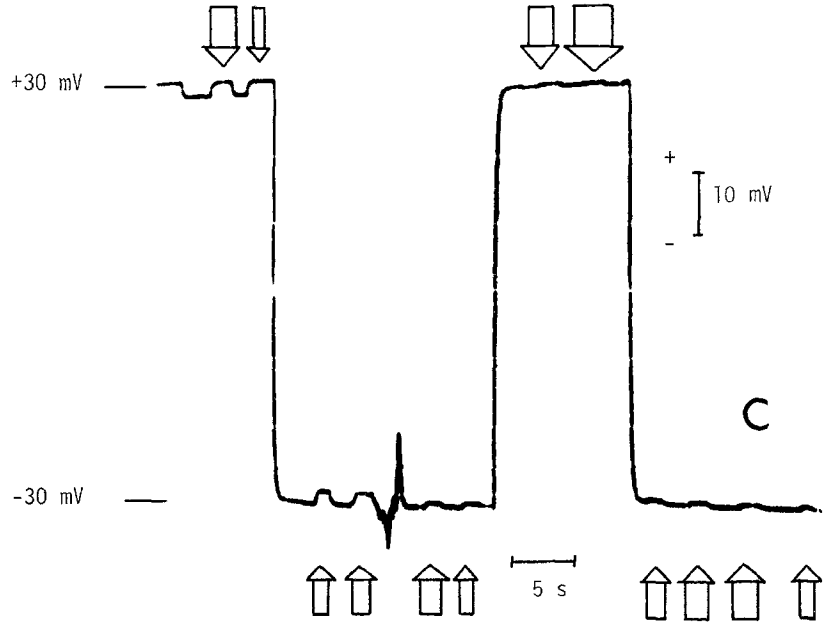
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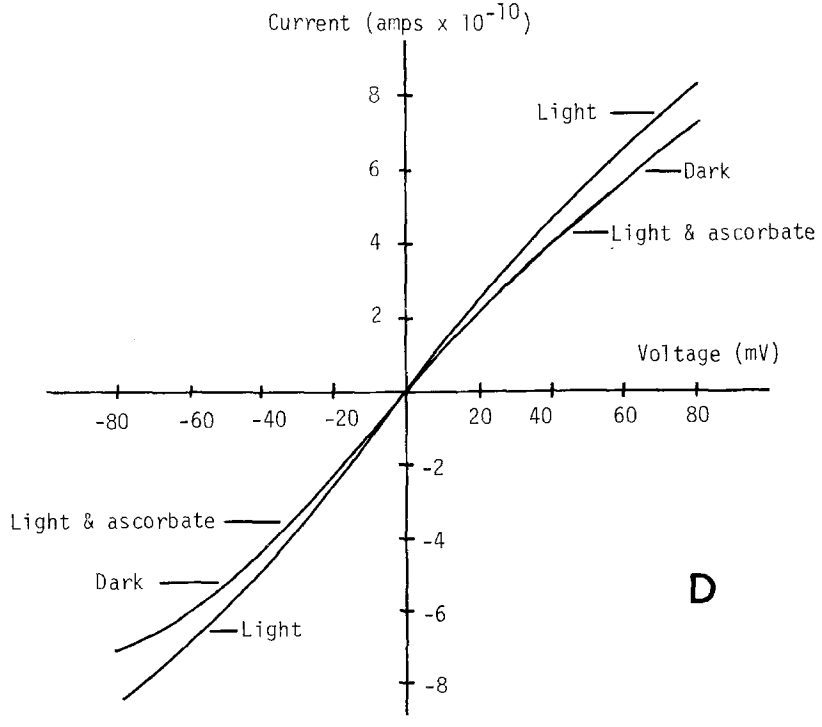
Current (amps x 10^{-10})
8
6
4
2
-2
-4
-6
-8
Light
Dark
Light & ascorbate
Voltage (mV)
-80
-60
-40
-20
20
40
60
80
Light & ascorbate
Dark
Light
D

Figure 3. Quencher effect upon pigmented BLM photoresponses. (A) Top trace: immediate elimination of the Slow Component after addition of 10^{-3} M ascorbate (indicated by noise); bottom trace: gradual disappearance of the slow transient in the presence of 10^{-4} M ascorbate. (B) Traces 1 and 3: biphasic responses in the absence of quencher; Traces 2 and 4: reduced Slow Component in the presence of 2×10^{-3} M NaN_3 in trace 2 and 2×10^{-3} M ascorbate in trace 4 where no Slow Component appears, only RC decay. (C) Quenching by 10^{-3} M ascorbate in the presence of repeated flash illumination ($\sim$50 Hz). Equal applied voltages of opposite polarity provide the only asymmetry across the Chl-BLM. The noise spike indicates introduction of quencher; subsequently the Chl-BLM is rendered relatively light-insensitive. (D) Light and dark current-voltage relationship of the Chl-BLM prior and subsequent to the addition of 10^{-3} M ascorbic acid.

decay process than attainable using single flash excitation. Clearly, the rates are not the same. The rate constant for this decay, lambda (λ), was typically 2 sec^{-1} while the RC rate constant was 5 sec^{-1}. (This corresponds to a 200 ms "RC time".) Though λ varied from one Chl-BLM to another within values of 0.5 to 2.5 sec^{-1}, for any single membrane λ remained constant and was not a function of time, applied voltage, duration of illumination or membrane resistance values.

Of a large variety of componds that had been tested on the Chl-BLM photosystem [6], several were found to eliminate completely or "quench" the pH or voltage-induced Slow Component without modifying the Fast Component. In order of decreasing quenching effectiveness, some of these compounds thus far tested are ascorbic acid, tannic acid (dicatechin), p-benzoquinone, and sodium azide. These compounds exhibited identical quenching properties in either one or both chambers. Ascorbic acid at 10^{-3} M eliminated the Slow Component immediately upon addition prior to stirring, as seen in Fig. 3A, while at 10^{-4} M 20 seconds of stirring was necessary in order for a similar degree of quenching to be observed. Fig. 3b compares the relative quenching efficiencies of ascorbic acid and sodium azide. The quenching phenomenon was essentially irreversible for any single BLM; i.e., once quenched the Slow Component did not reappear. Fig. 3c illustrates the ability of ascorbic acid to quench Slow Components of both polarities. These results were independent of which chamber contained the quenching agent. Further, the light and dark current-voltage characteristics of Chl-BLM in the presence and absence of Slow Component quencher were measured and are shown in Fig. 3d. As indicated, after the addition of quencher the I/V characteristics of the membrane were idential in both the dark and upon illumination.

Discussion

As an ultrathin ($\sim$100 Å), relatively large surface area, low dielectric constant partition between highly conducting aqueous phases, the

Chl-BLM possesses an appreciable electrical capacitance, usually $\sim 5 \times 10^{-9}$ F (C_m), and electrical resistance of 1×10^7 to 5×10^8 ohm (R_m). Electrically, the BLM is equivalent to a capacitor shunted with a resistor (i.e., a "leaky" capacitor). As any voltage is applied to this system, either through an external voltage source of via the action of light, the voltage transients appearing across the effective membrane R_m-C_m combination obey the following relationship:

for charging

$$V(t) = V_o [1 - \exp(-\frac{t}{RC_m})] \qquad [1]$$

and for discharging

$$V(t) = V_o [\exp(-\frac{t}{RC_m})] \qquad [2]$$

where V_o is the maximum voltage appearing across the BLM, $V = Q/C_m$ and R is the Thevenin equivalent resistance with respect to C_m. For the Chl-BLM this value is given by R_m in parallel with the internal or series resistance of the EMF shunted across the membrane (i.e., $R = R_s R_m/(R_s + R_m)$). In the simple case of a voltage discharge when the charging element has been removed, R_s is nearly infinite and thus $R = R_m$ and the discharge rate constant is simply $1/R_m C_m$, typically 5 sec^{-1}. For a potential applied through a resistance ($R_s = 10^8$ ohm) R becomes 5×10^7 ohm ($R_m = 10^8$ ohm) and the charging rate constant equals 10 sec^{-1}.

The Chl-BLM flash-elicited voltage in the case of an imposed transmembrane chemical asymmetry indicates rapid negative charge flow (<1 ms) to the $FeCl_3$/Chl-BLM side of the biface thereby charging the BLM capacitance. This is followed by the total disappearance of charging current flow resulting in discharge of the membrane capacitance in precisely the same kinetic manner as C_m is discharged in the dark according to membrane $R_m C_m$ characteristics. The rapid initial phase of the voltage transient represents charge separation across the membrane by a current-delivering element not hindered by high internal resistance. The condition in which charge separation can be established across the Chl-BLM capacitive element within only 1 ms is that the imput Thevenin equivalent resistance be reduced to less than 10^5 ohm, yielding an RC charging time of less than 0.5 ms. The experimental decay of the Fast Component is seen to be kinetically identical to the $R_m C_m$ transient exhibited by the Chl-BLM in the dark when the charging element is suddenly removed (cf. Fig. 2a).

Measurements of the equivalent internal resistance of the $FeCl_3$-induced photo-EMF under flash illumination were carried out by establishing the value of external shunt resistance, R_s, capable of

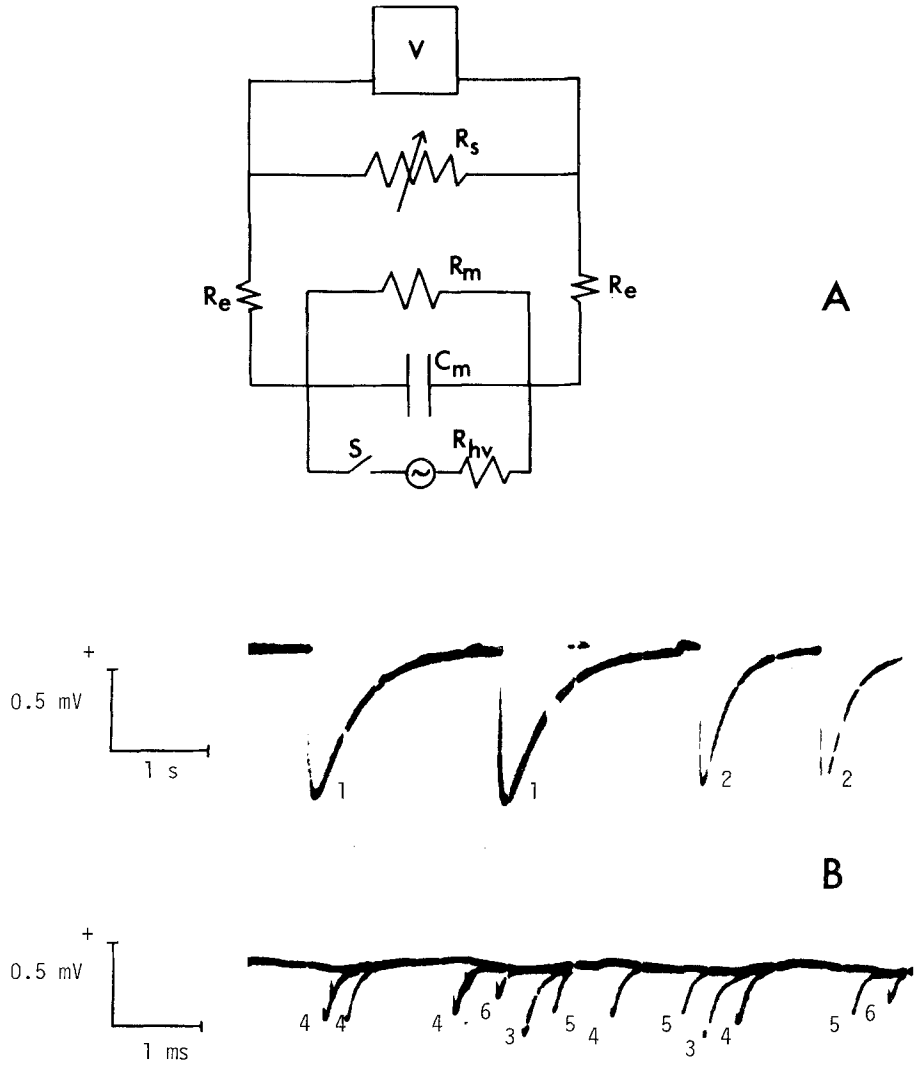

Figure 4. Equivalent circuit characterization of the Chl-BLM and the experimental effect of low external shunt resistance upon the $FeCl_3$-induced Fast Component. The flash response is represented as a microsecond closure of switch S. R_S equals: 1. 1.0×10^9 Ω (10%); 2. 9.1×10^7 Ω (10%); 3. 5.4×10^4 Ω; 4. 4.0×10^4 Ω; 5. 3.0×10^4 Ω; 6. 2.0×10^4 Ω.

reducing the absolute magnitude of the Fast Component to 50% of its open-circuit value. Under these conditions R_s matches $R_{h\nu}$ according to the representation in Fig. 4 since half of the photoemf is dropped across $R_{h\nu}$. As indicated in Fig. 4, $R_{h\nu}$ is on the order of 5×10^4 ohm. The electrodes (R_e) in series with R_s present resistances of approximately this magnitude and thus provide a lower limit on possible shunt resistance. Note the extremely short RC time constants as expected for Thevenin equivalent resistances reduced to this extent.

This analysis, then, indicates that a brief transmembrane current of nearly 10^{-8} amp flows to the membrane capacitance upon flash excitation in the case of $FeCl_3$-induced Fast Components. These results support the view that, the chlorophyll molecule excited by light to a reduction potential more negative than that of the Fe^{+3}/Fe^{+2} reduction-oxidation couple, may donate an electron to the ferric (Fe^{+3}) ion, the oxidized chlorophyll subsequently being reduced by substrate, most likely H_2O, at the biface [4]. These coupled oxidation-reduction reactions driven by the photoenergy harvested by the Chl-BLM imply a net electron flow to the $FeCl_3$/Chl-BLM interface. After illumination and the charging of C_m due to this current flow, the decay process follows $R_m C_m$ exponential decay kinetics, indicating that light-stimulated redox processes have ceased; indeed, the capacitance dissipates this accumulated charge precisely as it does any charge added to it in the dark, presumably via ionic flow. In addition, these decay kinetics illustrate that the membrane capacitance becomes charged and thus the initial photo-stimulated charge flow must be a transmembrane one, not merely an electrical double-layer charging current localized at a single interface.

Transient responses in the case of pH increments across the membrane displayed waveforms kinetically identical to applied voltage responses; therefore applied voltages were employed in the majority of experiments investigating the Slow Component, eliminating several limitations and precautions necessary in the pH difference instances, viz.: applied voltages were easily varied or removed and reapplied during experiments while pH asymmetries could not so easily be varied or eliminated; it has been shown that Chl-BLM functions as a hydrogen electrode, exhibiting the hydrogen ion Nernst potential in the dark [4], and this "dark potential" invariably introduced a voltage bias requiring difficult normalization of data; and also pH differentials were likely to result in membrane surface asymmetries.

Initially, the Slow Component's decay process was examined. As pointed out earlier, the rapid repeating flash approach was optimal for this study as it provided a large amplitude photoresponse concomitant with long-lived easily-resolved decay. It will be shown later that the decay form is not altered by the number of flashes incident upon the BLM prior to decay. The results are indicated in Fig. 5. Significantly, the

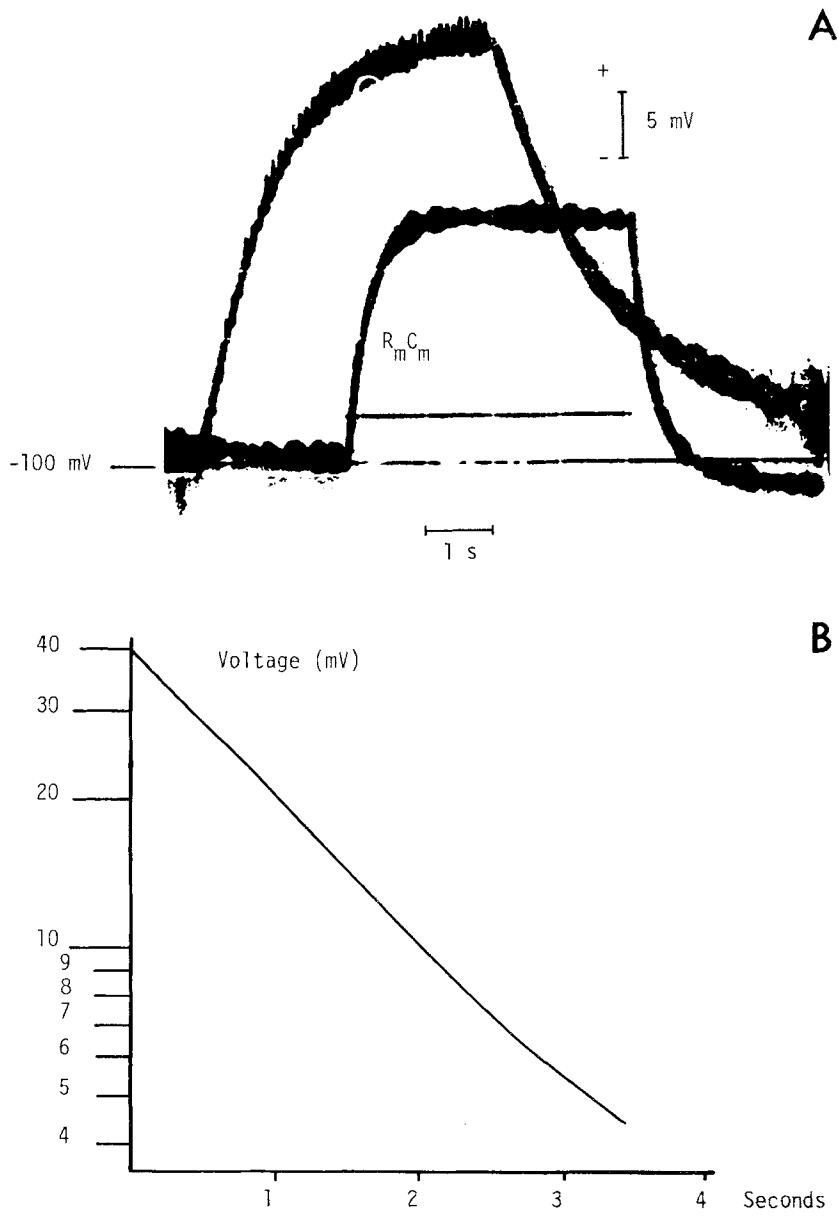

Figure 5. Experimental Slow Component decay (λ) and plot of log V values versus time. The linearity of this plot demonstrates the first order exponential character of the decay. (RC decay is again included for purposes of comparison.)

decay process was found to be a simple first order exponential one. That is, for the beginning of decay at t = 0,

$$V(t) = V(0)\exp(-\lambda t) \tag{3}$$

where λ is the rate constant. Although for different Chl-BLM λ varies from 0.5 sec^{-1} to 2.5 sec^{-1}, corresponding to 1/e times (duration in which V falls to V/e or 0.368V) of 200 ms and 400 ms, respectively, λ remains essenti· lly constant for any single BLM.

A major point is that λ is not equal to, or a function of, $R_m C_m$. It has been found that λ is always greater than $(R_m C_m)^{-1}$. In other words, λ decay is always slower than $R_m C_m$ decay, as shown in Figs. 2c and 5. If $R_m C_m$ decay is assumed, it results in the problem that the theoretical voltage falls too rapidly after the peak value.

Additionally, if the initial phase of the response is assumed to be a first order exponential process, the voltage-time course in the absence of decay and for strobe flash at t = 0, is given by

$$V(t) = V_o [1 - \exp(-Kt)] \tag{4}$$

where V_o is the voltage value at $t = \infty$. Whether this assumption holds for experimental waveforms can be determined in the following manner. If the initial phase is first order exponential,

$$V(t) = V_o [1 - \exp(-Kt)] \exp(-\lambda t) \tag{5}$$

will accurately describe the entire waveform. The constant λ was measured experimentally and K was determined numerically for experimental waveform sets of values of V and t. This computation was facilitated by taking the first derivative of V(t) with respect to time and measuring the experimental time interval from t = 0 to the moment of peak voltage, t_p.
Thus

$$K = \lambda [\exp(Kt_p) - 1]. \tag{6}$$

Now for any t,

$$V(t) = V_o [1 - \exp(-Kt)] \exp(-\lambda t) \tag{7}$$

yields a voltage value that may be compared with the experimental voltage at that instant. The fit achieved is quite good, as shown in Fig. 6a. The fact that lambda values derived from rapid repeating flash illumination provide accurate fits to single flash waveforms is clear indication that the decay form is not altered by the number of flashes incident upon the membrane prior to decay.

In the course of verifying this Slow Component fit for several Chl-BLM, it was observed that the constant K for any particular membrane was invariably equal to $(R_m C_m)^{-1}$ for that BLM. This observation introduced distinct advantages: (a) the numerical method for solving for the value of K could be abandoned; (b) Chl-BLM Slow Component waveform shapes could be predicted exactly, prior to any

flash illumination with λ having been measured from continuous illumination response decay; $K = (R_m C_m)^{-1}$ having been determined in the dark, as shown in Fig. 6a.

Furthermore, from equation [7] setting

$$\frac{dV(t)}{dt} = 0 \tag{8}$$

when $t = t_p$, yields

$$t_p = \ln \left[\frac{K + \lambda}{\lambda}\right] / K \tag{9}$$

which expresses the voltage waveform peak time in terms of λ and $(R_m C_m)^{-1}$. As a test of this prediction, external resistances were shunted across the Chl-BLM, effectively changing R_m and consequently also t_p. Fig. 6b indicates experimental peak times altered precisely as expected. The successful prediction of experimental waveform shapes justifies the original assumptions and provides a basis for identification of the photo-stimulated membrane events responsible for the Chl-BLM Slow Component response to flash illumination. The current-voltage relationships of the membrane in the dark and upon illumination (cf. Fig. 3d) together with the fact that the initial growth phase of the Slow Component exhibits a $(R_m C_m)^{-1}$ rate constant provide support that the Slow Component is a result of Chl-BLM conductivity enhancement upon illumination. All of the results are in accord with this interpretation.

That this conductivity enhancement or resistance decrease is 3% at most accounts for the fact that $R_m C_m$ values measured in the dark were sufficiently close to the slightly ($<3\%$) smaller $R_m C_m$ values actually characteristic of the membrane upon illumination that excellent fits were obtained regardless. Note in Fig. 6a that the experimental voltage increase is very slightly more rapid than the rise predicted according to the dark $R_m C_m$ values. The sudden introduction of slight additional conductivity across the membrane simply decreases the voltage dropped across the BLM, whose resistance in relation to the fixed shunt resistance determines the amount of transmembrane potential. This transmembrane potential decrease would be expected to display $R_m C_m$ initial phase kinetics. The lambda decay of the Slow Component, the first order exponential return to the dark resistance, is slower than the $R_m C_m$ transient and hence is not at all modified by the membrane $R_m C_m$ filtering capacity.

As discussed above, the quenching phenomenon represents an elimination of light-induced conductivity enhancement. An ascorbate concentration versus photoconductivity quenching ability profile is given in Fig. 6c. Since prior to quencher addition the enhanced conductivity decays with rate constant λ of $\sim 2\ \text{sec}^{-1}$, a metastable state of lifetime

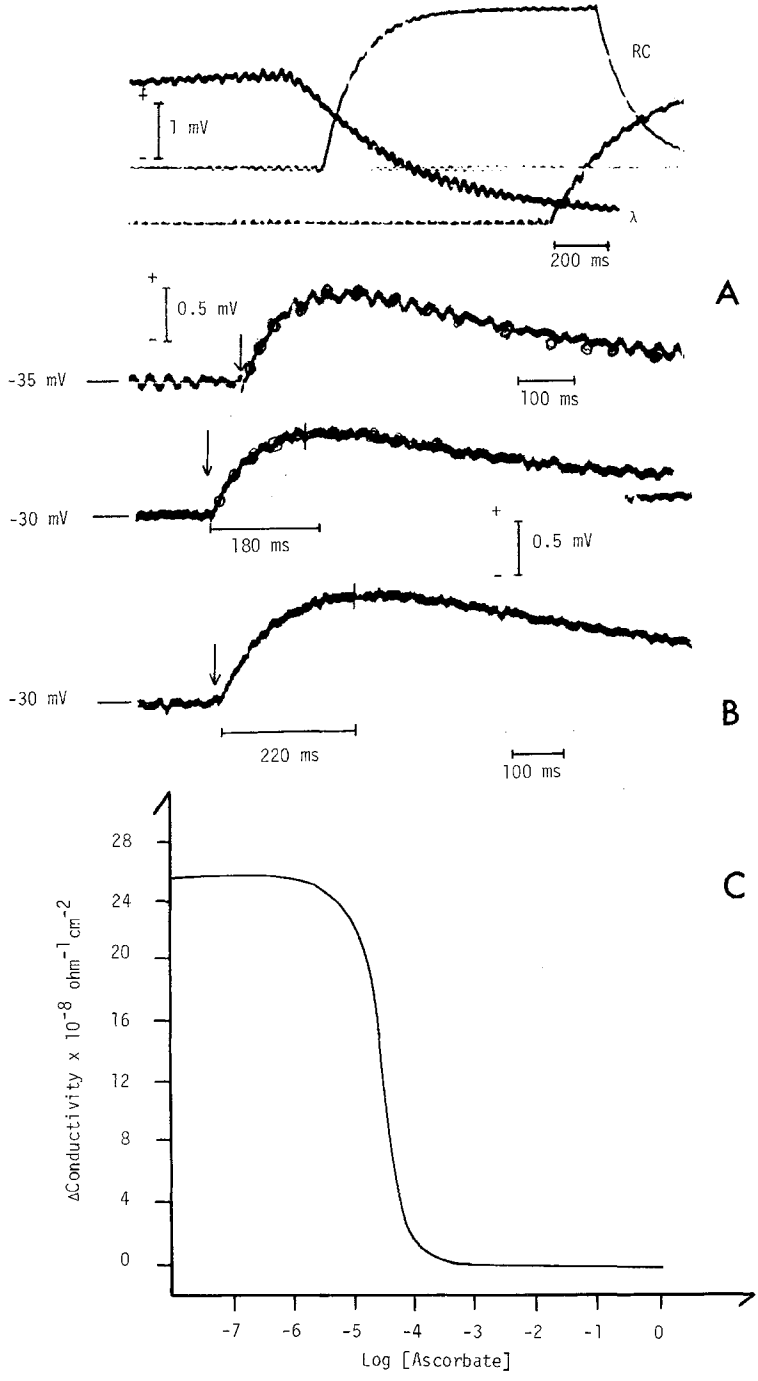
RC
1 mV
λ
200 ms
A
0.5 mV
-35 mV
100 ms
-30 mV
180 ms
0.5 mV
B
-30 mV
220 ms
100 ms
C
ΔConductivity x 10⁻⁸ ohm⁻¹cm⁻²
28
24
20
16
12
8
4
0
-7
-6
-5
-4
-3
-2
-1
0
Log [Ascorbate]

Figure 6. Theoretical fit to experimental waveforms and photoconductivity dependence upon quencher concentration. (A) Slow Component waveform and points calculated using RC and λ data included in figure. (B) Slow Component peak time, tp, dependence upon effective Rm. In both cases $tp = \ln[(K + \lambda)/\lambda]/K$, $C_m = 3 \times 10^{-9}$ F and $\lambda = 1.9$ sec^{-1}. (A) $R_m = 3.21 \times 10^7$ Ω ($R_S = 9 \times 10^7$ Ω); calculated tp = 180 ms. (B) $R_m = 4.76 \times 10^7$ ($R_S = 1 \times 10^9$ Ω); calculated tp = 221 ms. (C) Transient BLM photoconductivity dependence upon quencher concentration. Dark conductivity = $\sim 1.5 \times 10^{-6}$ ohm^{-1} cm^{-1}.

$\sim$500 ms is likely responsible. Recognizing that the conductivity enhancement action spectrum correlates with the chlorophyll absorption spectrum, that the chlorophyll triplet excited state may possess lifetimes of this duration [8], that Slow Component enhancer Fe enhances also excited singlet to triplet intersystem crossing, that the Slow Component quencher ascorbic acid is reported to be an excellent in vitro chlorophyll triplet quencher [7], and that at concentrations of chlorophyll likely in the Chl-BLM chlorophyll triplet states would be expected to decay in a first order exponential manner [8], it is reasonable to suppose that photo-populated chlorophyll triplet states are responsible for the conductivity enhancement in the Chl-BLM manifested as the Slow Component.

The Fast Component appears to represent a current-delivery process at a polarity fixed only by the arrangement of redox agents; the Slow Component is likely a consequence of a conductivity increase (e.g., via an exciton dissociation process) with kinetic features containing information concerning chlorophyll metastable states in the Chl-BLM.

Acknowledgments

This work was supported by a NIH training grant (GM-01422) and grant (GM-14971) from the National Institutes of Health. Parts of this paper are derived from the M.S. Thesis of T.E.M. completed in December 1973.

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