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Dependence of Photosensitivity of Bileaflet Lipid Membranes upon the Chlorophyll and Carotenoid Content

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Summary. Bileaflet lipid membranes were formed from solutions containing lecithin, chlorophyll and carotene in various concentrations. If all the above components were present at sufficient concentrations the membranes were photosensitive; i.e., a photocurrent was produced if a redox potential gradient was present across the membranes. The presence of chlorophyll and carotene were essential for the photosensitivity of the membranes. Photoresponse could be elicited by illuminating the membrane with light which did not excite carotene. On the other hand, elimination of the part of the light spectrum which excites chlorophyll led to the abolition of the photoresponse. The findings of this study are consistent with the assumption that the excited chlorophyll chromophores allow electron exchange at the membrane-water interface while the presence of carotene allows electron movement across the "bulk" lipid membrane.

In recent years, many experiments studying the nature of photosensitivity in bilayer lipid membranes (BLM) were performed [4, 12]. Often, a redox potential gradient was needed for the observation of the photoresponse. The lipid used in many of these studies was extracted from one of a number of plant sources [4, 12, 13]. The composition of the extract in terms of lipids and pigments was not accurately known. It was shown that the action spectra of the photoresponse followed the absorption spectrum of chlorophyll [4, 12] and the ability of an extrinsic membrane protein to direct electron flow across the membrane-solution interface was also demonstrated [4, 5]. A general theoretical model based on semiconductor electrochemistry [2] has been successful in explaining many of the observed effects [5].

However, none of the experimental studies dealt directly with the question of molecular functions of chlorophyll and carotenoids in the electron transfer process. It was suggested [4] that chlorophyll is necessary for the

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production of electrons and holes for interfacial electron transport processes while carotenoids are needed to provide a low resistance transmembrane electron pathway.

To demonstrate experimentally the functions of chlorophyll and carotenoids, a synthetic approach is necessary. This involves starting with a lipid and adding pigments in various mixtures and concentrations until the pigment requirements for a photoresponse are determined. Once the pigment requirements are known, action spectra of the photoresponse may help in analyzing the functions of the various pigments. This paper represents the results of such a study.

Materials and Methods

Membranes were formed by the brush technique [8] on a small hole in a teflon cup which rested in a larger lucite chamber. Membrane formation was monitored by measuring the capacitance and conductance of the membrane with a General Radio Z-Y bridge. As soon as lipid was placed on the hole, the conductance fell to a very small value. In the ensuing time (1 to 15 min, depending on solvent), the capacitance increased and stabilized. To accurately measure the resistance, a Keithley 601 electrometer was connected in place of the bridge. A small current was passed, via a second set of electrodes, through the membrane and the voltage developed was measured by the electrometer. All the electrodes were silver-silver chloride. Contact with the solution was made through 3 M KCl agar bridges. Photovoltages were measured with the electrometer and recorded by a Servoger recorder. Photocurrents were calculated by dividing the photovoltage by the membrane resistance. The validity of this computation was demonstrated in a previous article [4]. A normalization factor for membrane area was included. Membrane area was calculated using a specific capacitance of 0.38 $\mu F/cm^2$ [3]. Any error introduced by this particular value is systematic and not extremely important.

Membranes were formed in solutions of 0.1 M KCl containing 0.1 to 1.0 mM $FeCl_2$ and 0.1 to 1.0 mM $FeCl_3$. After the membrane had formed, a small volume of concentrated $FeCl_3$ was added to the inner cup, establishing the redox potential gradient. The final ferric concentration in the inside cup was 10 to 15 mM. A potential difference of about 60 mV developed due to the decrease in pH in the inner cup brought about by the addition of $FeCl_3$. A current was passed through the membrane to insure a base-line potential of zero.

Illumination was provided with an Edmund Scientific illuminator and fiber optics light guide. The intensity of illumination was controlled by using a variable power supply. Periodically, the intensity was checked with a Radiometer (Model G 5-Yellow, Spring Instrument Co.). The intensity at the membrane surface was 120 to 150 mW/cm^2, comparable with previous values [4]. The illuminator contained its own shutter. The light guide diameter was about six times that of the membrane diameter and thus the whole membrane was illuminated.

Phosphatidyl choline was obtained from Sigma (egg yolk, prepared chromatographically). The chlorophyll, in a paste form, was obtained from K & K Laboratories and contained roughly 0.37 g chlorophyll/g paste. Chlorophyll-a from Sigma is free from chlorophyll-b *but contains xanthophyll.* However, the two pigments may be separated by thin-layer chromatography [11]. In a few experiments, the purified xanthophyll-free chlorophyll-a was used instead of the K & K extract. Similar results were obtained.

Another precaution which had to be taken was to use phosphatides free of yellowish pigments. Some of the preparations of phosphatides, especially the phosphatidyl serine, has marked absorption at the 400 to 500 nm range. With those "impure" phosphatide preparations the photoresponse could be elicited in "carotene-free" membranes.

Carotene was obtained from Eastman Kodak. Xanthophyll was obtained from Nutritional Biochemicals and was purified by running a benzene solution of xanthophyll on a $CaCO_3$ column [6]. Afterwards, the xanthophyll was eluted with *t*-butanol.

The membrane-forming solution was prepared by mixing stock solutions of phosphatidyl choline, chlorophyll and carotene (all dissolved in *t*-butanol) with hexane. The final hydrocarbon concentration in the membrane-forming solution was 20 to 25% by volume. The final concentration of phosphatidyl choline was generally 3 mg/ml. The concentrations of chlorophyll, carotene and xanthophyll were varied as specified for each experiment.

It should be noted that the absorbance of the membrane-forming solution due to chlorophyll in the previous work was comparable to the maximal absorbance of the membrane-forming solution due to chlorophyll in this study.

Cut-off filters were obtained from Central Scientific Company. Transmittance characteristics of the filters and absorption spectra of the membrane-forming components were measured with a Cary 14 spectrophotometer.

Results

The pigment requirements for the photoresponse are shown in Fig. 1. As is seen, a BLM made of phosphatidyl choline (*a*), phosphatidyl choline + chlorophyll (*b*), or phosphatidyl choline + carotene (*c*) did not show a photovoltage response upon illumination. However, it is clearly seen that a membrane made of phosphatidyl choline, chlorophyll and carotene (*d*) did show a substantial photoresponse. It seems that both chlorophyll and carotene were needed for the photoresponse. Purified xanthophyll could be substituted for carotene at equivalent concentrations and similar results were obtained.

The photovoltage response in the presence of different filters is shown in Fig. 2. With an orange (#2) or red (#4) filter, the photoeffect was comparable to the photovoltage with no filter. However, with blue and green (# 1 and # 3) filters, the photoeffect was below the limit of detection.

Thus, the demonstrated necessity of carotene for a photovoltage response was not associated with its properties as a light-absorbing pigment. On the other hand, when the light which was absorbed by the chlorophyll red peak was removed, there was no observable photoresponse.[1]

1 The blue peak (410 nm) of chlorophyll did not contribute to the photoeffect in our system due to two reasons: (a) the intensity of the incident light was very low in the blue range (*see* Fig. 10 in Ref. [4]) and (b) the ferric solution in the outer cup acted like a blue light filter (*see* Fig. 12 in Ref. [4]).

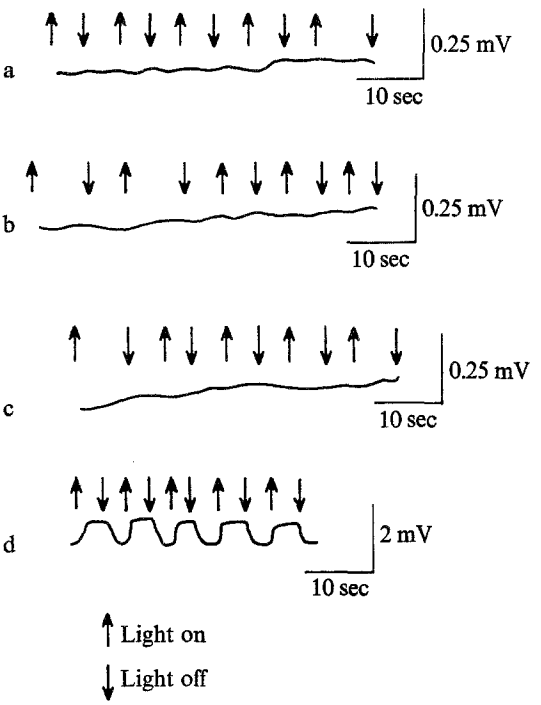

Fig. 1. The pigment requirements for a photoeffect. The concentration of phosphatidyl choline was constant, 3 mg/ml, throughout the series of experiments. The solvent was a mixture of *t*-butanol and hexane (3:1). (*a*) Phosphatidyl choline BLM (500 MΩ, 4000 pF); (*b*) phosphatidyl choline and chlorophyll (4.5 mg/ml) BLM (400 MΩ, 3400 pF); (*c*) phosphatidyl choline and carotene (0.19 mg/ml) BLM (300 MΩ, 3600 pF); (*d*) phosphatidyl choline and chlorophyll (1.11 mg/ml) + carotene (0.16 mg/ml) BLM (300 MΩ, 3200 pF). Note that only when carotene and chlorophyll were present in the membrane-forming solution there was a clear photovoltage response. The basic aqueous solution was 0.1 M KCl. The inside solution also contained 16 mM $FeCl_3$ + 1 mM $FeCl_2$; the outside solution 1 mM $FeCl_3$ + 1 mM $FeCl_2$. The inside cup became negative with respect to the outside cup upon illumination (in *d*)

Experiments using interference filters for the red range produced an action spectrum of the photovoltage response which followed the chlorophyll absorption and was similar to previous results [4].

Figs. 3 and 4 show the dependence of photocurrent upon the chlorophyll and carotene concentrations, respectively. Generally, the higher the concentration of both pigments, the higher the photoeffect. It is interesting to note, however, that while the photocurrent was a continuous function of the chlorophyll concentration it showed a "threshold" dependence on carotene content.

The relationship between the concentration of a species in the membrane-forming solution and its abundance in the bileaflet structure is unknown.

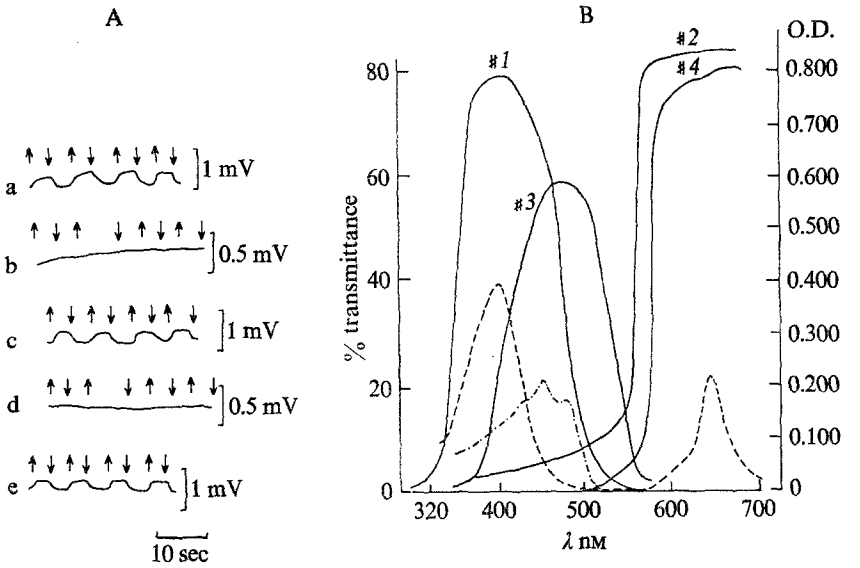

Fig. 2. (*A*) Photoresponse as a result of illumination through different filters. BLM made of phosphatidyl choline (3 mg/ml), chlorophyll (1.74 mg/ml) and carotene (0.16 mg/ml) (350 MΩ, 4000 pF). Other details as in Fig. 1. (*a*) No filter; (*b*) filter #1; (*c*) filter #2; (*d*) filter #3; (*e*) filter #4. The transmittance curves (full lines) of the filters are shown in Fig. 2(*B*). The absorbance of chlorophyll (2.2×10^{-3} mg/ml; dashed line) and β carotene (0.6×10^{-3} mg/ml; dashed and dotted line) solutions in *t*-butanol are also included. Note that elimination of the part of the spectrum which excites carotene (filters #2 and #4) did not interfere with the photoresponse, whereas elimination of the 680 nm range (filters #1 and #3) which excites chlorophyll abolished the photoresponse

However, it is plausible that for a lipid-soluble, nonpolar molecule like carotene the above relationship would be linear. For an amphophilic molecule like chlorophyll the above relationship would also be linear provided an excess of other amphophilic molecules (e.g., phosphatidyl choline) are present in the solution. Thus, at least for the lower range of chlorophyll concentrations shown in Fig. 3, it can be assumed that the chlorophyll density in the bileaflet membrane is proportional to its concentration in the bulk solution.

Fig. 3 shows a comparison of the ability of xanthophyll and carotene to produce a photoresponse. It is obvious that the effectiveness of both pigments in producing a photocurrent was comparable at high concentration. At lower concentration the β-carotene exhibited a "threshold phenomenon" while xanthophyll did not. The reason for this difference is not clear. It may be related to the difference in the surface activity of the two pigments.

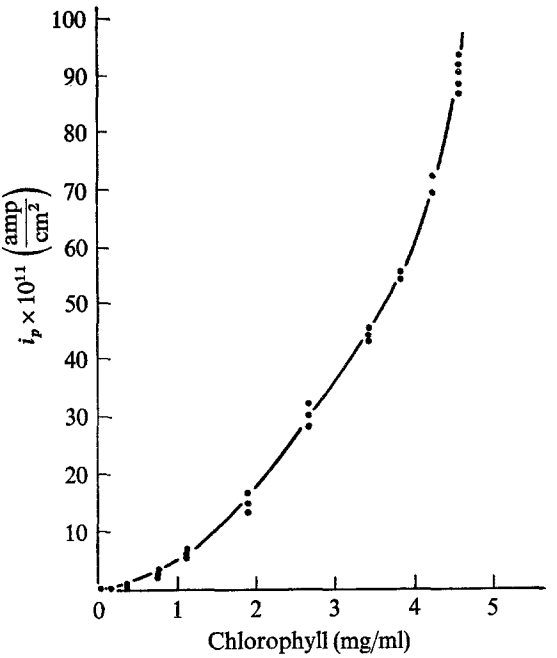

Fig. 3. The dependence of photocurrent on chlorophyll concentration in the membrane-forming solution. For all membrane-forming solutions the concentrations of carotene and phosphatidyl choline were 0.15 mg/ml and 3 mg/ml, respectively. Other details as in Fig. 1. Each point represents results of experiments performed on one membrane. For each membrane, a minimum of three responses were recorded. The photovoltage response was constant to within 10% of the average. The curve corresponds roughly to linear dependence of photocurrent on the second power of chlorophyll concentration

Discussion

The data presented in this paper are consistent with the assumption that both chlorophyll and carotenoids are essential for the photosensitivity of bileaflet lipid membranes made of chloroplast extract. According to the ideas presented in the previous papers [4, 5], the function of chlorophyll in the photosensitive membranes is to absorb the photons and thus, allow electron movement across the membrane-water interface. The function of carotenoids is to provide a pathway of low enough resistance to electron movement across the "bulk" membrane. The relative low resistance to electron flow is established by the long alternating double bond structure of the carotenoids [10].

The quantitative study of the dependence of the photocurrent on pigment content in the membrane-forming solution reveals the following features: (a) the photocurrent is proportional to the second power of chlorophyll

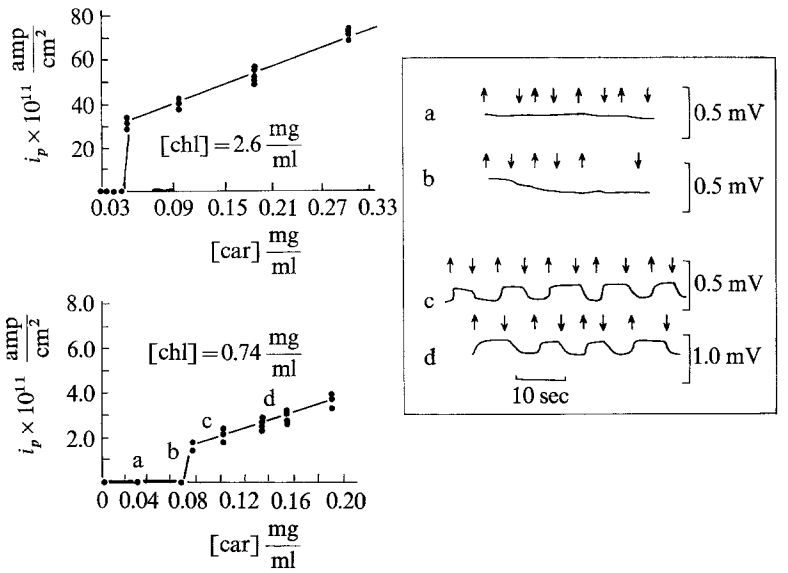

Fig. 4. The dependence of photocurrent on carotene concentration in the membrane-forming solution. Phosphatidyl choline concentration was 3 mg/ml. The concentrations of chlorophyll were 2.59 mg/ml (upper drawing) or 0.74 mg/ml (lower drawing). All other details as in Fig. 1. The inset on the right shows recording traces from which the values of the points *a*, *b*, *c* and *d* in the lower drawing were taken. For simplicity, the recording traces for other points are not shown. Each point represents results of experiments done on a single membrane. For each membrane, a minimum of three photoresponses was used. The photovoltage response for a particular membrane was constant to within 10%

concentration at constant β-carotene concentration (Fig. 3); (b) the photocurrent is proportional to xanthophyll concentration at constant chlorophyll concentration (Fig. 5); (c) the dependence of photocurrent on β-carotene concentration shows a threshold phenomenon. Above the threshold concentration of β-carotene the photocurrent increases linearly with the carotenoid concentration with a slope which is similar to that shown by the xanthophyll curve (Figs. 4 and 5). We do not have an explanation for the "threshold" phenomenon in the carotene curve.

The fact that the photocurrent is proportional only to the second power of chlorophyll concentration does not tally with the idea that large aggregates of chlorophyll are essential for the photosensitivity of the BLMs. One of the main features of the theoretical model suggested previously [5] is that assemblies of chlorophyll molecules can enhance the process of electron transport. It was suggested that such assemblies occur in the natural photosynthetic membranes and that the lack of such assemblies in the BLMs is responsible for the very low quantum efficiency (i.e., electron transport per

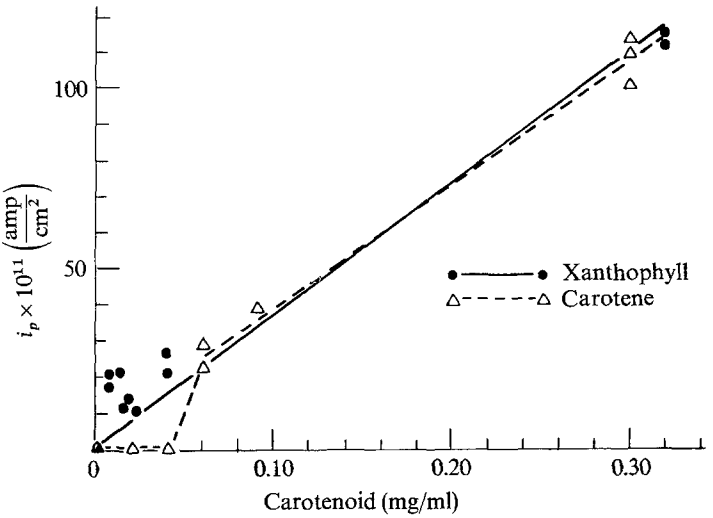

Fig. 5. A comparison of the effectiveness of xanthophyll and carotene in causing photosensitivity of BLMs. The BLM was made from a solution containing phosphatidyl choline (3 mg/ml) + chlorophyll (3.25 mg/ml) + varying concentrations of carotene (△ – △) or xanthophyll (● – ●). The basic aqueous solution was 0.1 M KCl. The inside solution contained also 15.1 mM $FeCl_3$ + 0.1 mM $FeCl_2$. The outside solution contained 0.1 mM $FeCl_3$ + 0.1 mM $FeCl_2$. Thus, this figure and the preceding ones are not directly comparable. Each point represents the results of experiments performed on one membrane. For each membrane, a minimum of three responses were recorded; the photovoltage response was constant to within 10% of the average

excited electrons) of the photocurrent observed in these membranes [5]. It was not possible to raise further the chlorophyll concentration in the experiment reported in this study because of the limit of the chlorophyll solubility in the particular solvent used.

It is worthwhile to note that the upper limit of the photocurrent observed in the membranes of this study was 10^{-9} amps/cm^2, comparable to the photocurrent observed in chloroplast extract membranes of the previous study [4]. The optical density due to chlorophyll was similar in both sets of experiments. Our conclusion would be that in both cases there are no assemblies of chlorophyll to any significant degree.

While it is possible that a more detailed study may reveal some other lipidic molecules necessary for the enhancement of photocurrent in BLM it seems to us that the next main steps toward finding factors which can increase the quantum efficiency of BLM photocurrent are likely to be revealed by: (1) manipulation of the nature of electron acceptors and donors in the

aqueous solution which affect the energy barriers to electron exchange at the interface, and (2) construction of bileaflet membranes devoid of solvents, such as those described by Montal and Meuller [7].

The latter point is of particular interest since the presence of the organic solvents in BLM [9] may be the main cause for the lack of pigment aggregates necessary for the facilitation of electron exchange at the membrane-water surface.

According to Fettiplace, Andrews and Haydon [1] BLM made from a solution of long hydrocarbons such as *n*-hexadecane "contain effectively no solvent". In some of our experiments we have substituted *n*-hexadecane for *n*-hexane. This did not have any drastic effect on the intensity of the photocurrent. This may indicate that either the presence of *n*-hexadecane in the membrane is sufficient to prevent the formation of "effective" chlorophyll aggregates or that some other ingredients present only in the photosynthetic membranes are needed for the formation of such aggregates.

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