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RELATIONSHIP OF THE PHOTOSENSITIVITY OF BILAYER LIPID MEMBRANES AND THE AQUEOUS ACCEPTOR

STUDIES USING COMPLEX IONS OF AMINO ACIDS

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SUMMARY

The photocurrent in photosensitive bilayer lipid membranes has been studied as a function of the aqueous acceptor. Correlations are observed between the relative photocurrent and the position of the complex ion visible absorption band and the dipole moment of the ligand. The effect of the ligands is nondirectional: they may be added to either side of the membrane with a corresponding effect on the photocurrent. The effects of the ligands are interpreted using an energy barrier model.

INTRODUCTION

Bilayer lipid membranes which contain chlorophyll and carotene are photosensitive [1, 2]. If a photosensitive bilayer lipid membrane separates solutions of different redox potential, illumination causes charge movement and the production of a photopotential. It is believed that redox reactions occur at each membrane-solution interface. Ilani and Berns [2] showed that the rate of the redox reaction at the more oxidized interface could be increased if phycocyanin, an extrinsic photosynthetic protein, was added to the more oxidized solution. They also showed that Fe^{3+} had a specific effect on the fluorescence, which may indicate a complex of Fe^{3+} and phycocyanin [3]. Recently, it has been shown that any of a number of biliproteins can enhance the photocurrent in bilayer lipid membranes [4].

A theoretical explanation for the effect of phycocyanin on the photosensitive bilayer lipid membrane is that energy barriers to electron transfer are present at each membrane-solution interface [5]. When phycocyanin is not present, the height of the barrier is 4 eV. There are two mechanisms by which electrons can pass the barrier; hopping over or tunneling through. When the probabilities of transfer are calculated, the probability of tunneling is much greater than the probability of hopping

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[5]. Ilani and Berns suggested that phycocyanin lowers the interfacial potential barrier. When the barrier is lower, tunneling and hopping mechanisms have comparable probabilities and the photocurrent increases.

Aqueous ligands can greatly affect the rates of redox reactions in solution [6]. Since interfacial redox reactions may be responsible for the photocurrent in bilayer lipid membranes, it was conceivable that ligands complexed to aqueous donors or acceptors would affect the magnitude of the photocurrent. In this paper, we will report on the relationship between photocurrent and complex ions of various amino acids.

EXPERIMENTAL

Membranes were formed and photocurrents were measured as described previously [2, 7]. The composition of the membrane forming solution was 3 mg/ml phosphatidylcholine, 2.7 mg/ml chlorophyll and 0.2 mg/ml β-carotene. The lipid and pigments were dissolved in *tert*-butanol. In addition, hexane was present in the membrane-forming solution. The ratio of *tert*-butanol to hexane was always 3 : 1. Stock solutions of 10^{-1} M $FeCl_3$+10^{-1} M ligand were produced. The pH of the stock solutions was 3.0–3.4. A bilayer lipid membrane was formed in 10^{-1} M KCl+ 10^{-4} M $FeCl_3$+10^{-4} M $FeCl_2$. The solution of $FeCl_3$+ligand was added to one side of the membrane so that the final concentration of ligand was 10^{-2} M. The photovoltage response was recorded. The apparatus used for forming bilayer lipid membranes was cleaned and the experiment repeated using a different $FeCl_3$+ligand solution. This procedure was followed until all of the ligand solutions had been used. The photocurrent per unit area with $FeCl_3$+alanine on one side of the membrane was set equal to 1. All other photocurrents were expressed as the ratio of the photocurrent per unit area with a ligand to the photocurrent per unit area with alanine. All measurements were made with the dark membrane potential equal to 0 mV.

Optical absorption measurements were obtained using a Cary 14 with 1 cm cuvettes.

RESULTS

General effect

In Table I, the relative photocurrents are tabulated, as are the redox potentials of the stock solutions of complex ion. It is unlikely that the slight variations of redox potential of the stock solutions were responsible for the variations in photocurrent: the difference in redox potential between the two sides of the membrane was 50–100 mV for all experiments. In this region, a plot of photocurrent against redox potential has reached saturation. The data presented in Table I indicate that aqueous ligands affected the rate of electron transfer from the photosensitive membrane to the aqueous acceptor. In order to elucidate the mechanism of the effect, correlations between complex ion structure and the enhancement of the photocurrent were studied.

Optical absorption spectra and the photocurrent enhancement

When the amino acids were mixed with $FeCl_3$ solution a visible absorption band appeared in the 500–600 nm region. This absorption band was not observed

TABLE I

MODIFICATION OF BILAYER LIPID MEMBRANE PHOTOSENSITIVITY BY AQUEOUS LIGANDS

Various ligands were added to one side, which already contained 10^{-2} M $FeCl_3$. In the first column, the ligand which was added is listed. The number in parenthesis is the number of different membranes used to determine the enhancement of the photocurrent. In the second column, the relative photocurrent per unit area is listed. In the third column, the redox potential of the stock solution is given.

Ligand (10^{-2} M) in the inner cup*	Relative photocurrent	V_r(mV)**
None (18)	0.80 ± 0.08	764
Alanine (13)	1.00 ± 0.18	724
Phenylalanine (17)	1.34 ± 0.08	704
β-Alanine (17)	0.75 ± 0.23	694
Glutamine (20)	1.09 ± 0.23	714
Glutamic acid (18)	1.97 ± 0.18	704
Asparagine (17)	0.96 ± 0.13	714
Aspartic acid (16)	2.52 ± 0.20	724
Leucine (17)	1.39 ± 0.29	744
Methionine (10)	1.21 ± 0.16	704
Arginine (9)	0.73 ± 0.08	754
Glycine (11)	1.05 ± 0.08	724
Diglycine (11)	4.65 ± 0.48	754

* Each side of the membrane contained 10^{-4} M $FeCl_3$ + 10^{-4} M $FeCl_2$ + 10^{-1} M KCl. In addition, one side contained 10^{-2} M $FeCl_3$.

** Each side of the membrane contained 10^{-4} M $FeCl_3$ + 10^{-4} M $FeCl_2$ + 10^{-1} M KCl. The redox potential of this solution was 644 mV.

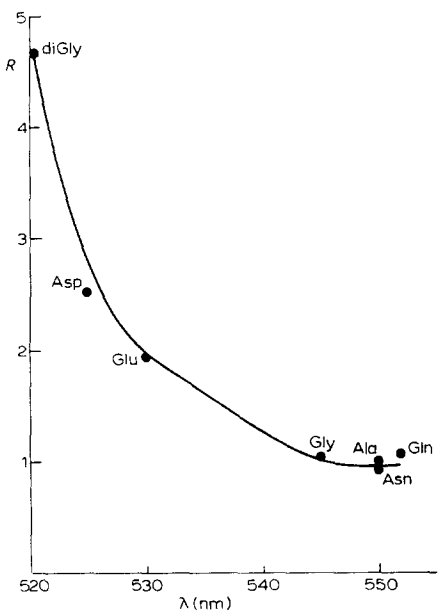

Fig. 1. The enhancement of the photocurrent caused by the addition of a ligand is plotted against the peak of the charge-transfer band of the complex ion. R is the relative photocurrent.

in the $FeCl_3$ or amino acid solutions alone and results from a mixing of the metal and ligand orbitals [8]. The properties of the new absorption band are specific to the complex ion.

In Fig. 1 the peak of the complex ion absorption band is plotted against the relative photocurrent. As the peak shifted towards a shorter wavelength, the photocurrent observed increased. A consistent correlation between the shape or size of the absorption band and the photocurrent was not observed.

According to the theory of Ilani and Berns, the photocurrent depends upon the overlap of the density-of-states bands of the membrane chromophore and the aqueous acceptor [5]. The position of the center and the bandwidth of the aqueous band are very difficult to calculate [9]. It is probable that the formation of the complex ion affects the position of the center and the bandwidth of the aqueous band. The results shown in Fig. 1 indicate that, as the peak of the absorption band of the complex ion shifted to shorter wavelengths, the overlap of the density-of-states bands increased. Quantum mechanical calculations which would complement the experiments appear to be impractical.

Electrical properties of the relative photocurrent

Ilani and Berns constructed a simple equivalent circuit model of the photosensitive bilayer lipid membrane [2]. They assumed that a "photobattery" was the driving force for the photocurrent and that a "photoresistance" exists. It was shown that from the slope of a plot of photovoltage response, ΔV, against dark membrane potential, V_m, the photoresistance could be calculated [2]. Also, the $\Delta V = 0$ intercept is approximately equal to the photobattery potential. Plots of photovoltage response against membrane potential were constructed using a number of ligands. Since the results were qualitatively identical, only experiments in which aspartic acid was used will be discussed.

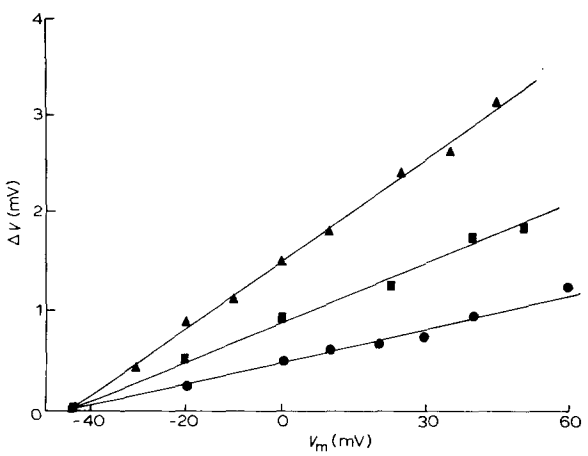

Fig. 2. Photovoltage response, ΔV, as a function of membrane potential. The ratio of the concentration of aspartic acid to the concentration of Fe^{3+} was 0.0 (●), 0.5 (■) and 1.0 (▲). Each side of the membrane contained 10^{-3} M $FeCl_2$ and 10^{-3} M $FeCl_3$. In addition, one side contained 10^{-2} M $FeCl_3$+aspartic acid. Each data point represents one measurement of the photovoltage response.

In Fig. 2, the photovoltage response is plotted against V_m for three concentrations of aspartic acid. The $\Delta V = 0$ intercept was about −45 mV for all three concentrations of aspartic acid. Thus, the photobattery driving force was independent of concentration of aspartic acid. As the concentration of aspartic acid increased, the slope of the ΔV vs V_m plot increased. We conclude that the photobattery resistance decreased. Following Ilani and Berns, we divide the photobattery resistance into an interfacial and an intramembrane resistance in series. It is unlikely that a polar amino acid such as aspartic acid could have an effect on the intramembrane resistance. It is probable that the interfacial resistance was lowered by the amino acid.

What is the mechanism by which the interfacial resistance was lowered? The ligand may allow closer approach of the acceptor to the membrane. Closer approach would facilitate more rapid electron transfer from the membrane to the acceptor. It is also possible that the ligand lowers the interfacial potential energy barrier to electron flow. For instance, the ligand dipole moment could interact electrostatically with the charges on the membrane surface. The interaction energy would then decrease the potential energy barrier.

Recently, an electrostatic theory which explains the effects of ligands on the rates of redox reactions has been developed [10]. It is assumed that the photocurrent is proportional to the probability of tunneling through the potential energy barrier. The relationship between photocurrent, I, and ligand dipole moment, μ, predicted by the theory [10] is

$$(\ln I)^2 = k_0 + k_1 \mu \tag{1}$$

where k_0 and k_1 are constants. In Fig. 3, the experimental values of the photocurrent are plotted against the ligand dipole moments, which were calculated according to Greenstein and Wintz [11]. The excellent agreement indicates that, in some cases, the ligands may enhance the photocurrent by lowering the potential energy barrier to electron flow via electrostatic interactions between the ligand and the donor charge [10]. The approximate decrease in the height of the energy barrier may also

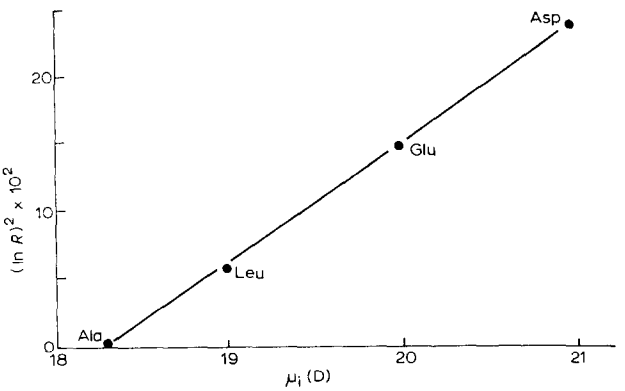

Fig. 3. Application of the modified theory to photosensitive bilayer lipid membranes. A plot of $(\ln R)^2$ against the ligand dipole moment. The probability of exceeding the linear correlation coefficient of the points shown by choosing random points from an uncorrelated sample is less than 0.0005.

be calculated using the electrostatic theory. In the case of the aspartic acid complex ion, the calculations indicate that the potential energy barrier is lowered by about 0.7 eV.

Ligands affect the photocurrent in two directions

The enhancement of the photocurrent caused by the addition of phycocyanin was unidirectional [2]. Phycocyanin caused an increase in the photocurrent only if it was added to the more oxidized side of the bilayer lipid membrane. The ligands used in this study did not exhibit the unidirectional property of phycocyanin.

In all of the experiments reported thus far, the gradient of redox potential was established by adding $FeCl_3$ to one side of the membrane. The redox potential gradient could also be created by adding $FeCl_2$ to one side of the membrane. The photocurrents in each system were comparable for comparable gradients of redox potential. If ligand+$FeCl_2$ was added to one side of the membrane, the photocurrent was higher than the photocurrent when ligand was absent. For example, the results for aspartic acid are:

Redox gradient established by	Ratio of Photocurrent with ligand present to photocurrent without ligand
Fe^{3+}	3.1
Fe^{2+}	2.2

Why did the aspartic acid increase the photocurrent by a smaller amount when the redox gradient was established by the more reduced species? Only a qualitative explanation is available presently. According to the energy barrier theory [5], the energy barrier to the transfer of an electron from the reduced species to the membrane is about 4 eV. The energy barrier to the transfer of an electron from the membrane to the aqueous acceptor is about 2 eV. The probability of tunneling through a potential energy barrier of height V is proportional to $\exp(-\sqrt{V})$. If the addition of the aspartic acid lowers the interfacial energy barrier by an amount Δ, the interfacial energy barriers will be $4-\Delta$ eV and $2-\Delta$ eV. The 2 eV barrier will be lowered by a larger fraction of the total barrier height than the 4 eV barrier. This result qualitatively explains the greater photocurrent when the redox potential gradient was established by the more oxidized species.

CONCLUSION

Aqueous ligands affect the photocurrent in photosensitive bilayer lipid membranes. We suggest that the ligands increase the rates of interfacial redox reactions that are responsible for the photocurrent. Various correlations between complex ion structure and the photocurrent were observed. Further research concerning these relations may help to elucidate the physical mechanisms involved in photosensitivity and, ultimately, photosynthesis.

ACKNOWLEDGEMENT

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