THE ENHANCEMENT OF PHOTOCURRENTS IN BILAYER LIPID MEMBRANES BY PHYCOCYANIN: pH AND SURFACE CHARGE DEPENDENCE

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SUMMARY

The enhancement of the photocurrent in photosensitive bilayer lipid membranes by phycocyanin was studied as a function of pH and membrane surface charge. As the pH increases, if the membrance surface charge is zero, the enhancement of the photocurrent increases. As the membrane surface charge becomes more negative, the enhancement decreases. If the surface charge is sufficiently high, no enhancement of the photocurrent occurs. These results are used to suggest some properties of the phycocyanin aggregate which enhances the photocurrent.

Ilani and Berns demonstrated that phycocyanin, an extrinsic energy transfer protein found in blue green algae, enhanced the photocurrent in a photosensitive bilayer lipid membrane (BLM) composed of chloroplast extract (1). Recently, it has been shown that a number of biliproteins can enhance the photocurrent (2).

The aggregation state of phycocyanin depends upon pH . At low pH , the 11S aggregate is predominant. As the pH increases, the amount of 6S aggregate present increases (3). It is probable, also, that the surface charge of the phycocyanin molecule changes with pH .

It is possible that knowing some of the properties of the phycocyanin aggregate involved in the enhancement of the photocurrent in BLM may help to elucidate the mechanism of the enhancement. Thus, the pH dependence of the enhancement of the photocurrent and the

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relationship between membrane surface charge and enhancement of the photocurrent were studied.

Experimental

Phycocyanin was provided by Dr. D.S. Berns (Division of Laboratories and Research, New York State Dept. of Health Albany, N.Y.). The methods used to form BLM and measure the photocurrent were those used in (4). Phosphatidyl choline, serine and ethanolamine were obtained from Sigma Chemicals. The concentration of phospholipid in the membrane forming solution was 3 mg/ml . The concentrations of chlorophyll and carotene were 3 mg/ml and 0.3 mg/ml , respectively. At pH 3 and 5 , the BLM were formed in 10^{-4}MFeCl$_3$ + 10^{-4}MFeCl$_2$ + 10^{-1}N Potassium Acetate buffer. At pH 6 and 7 , the BLM were formed in 10^{-4}MFeCl$_3$ + 10^{-4}Cl$_2$ + 10^{-2}MEDTA + 10^{-1}N Sodium Phosphate buffer. The EDTA was added to prevent precipitation of $Fe(OH)_3$. After formation of the BLM, $FeCl_3$ was added to one side of the membrane to increase the Fe^{+3} concentration to 1.01×10^{-2}M . The photovoltage response was measured at least three times and the photocurrent was calculated (4). Phycocyanin was then added to the side of the membrane which had the higher Fe^{+3} concentration. The concentration of phycocyanin was about 0.15 mg/ml . Thirty minutes after the addition of phycocyanin, the photocurrent was determined once more.

Results

In table 1 the results obtained using three phospholipids are shown. R is the enhancement of the photocurrent by phycocyanin:

$$R = \frac{\text{photocurrent with phycocyanin added}}{\text{photocurrent without physocyanin}}$$

The data concerning membrane surface charge were taken from Bangham (5).

Discussion

The results shown in Table 1 can be used to suggest properties

TABLE 1

Membrane Phospholipid	pH	Enhancement* by Phycocyanin	Relative Surface# Charge
Phosphatidyl-choline	3	1.25	0.0
	4	-	0.0
	5	1.75	0.0
	6	2.30	0.0
	7	2.90	0.0
Phosphatidyl-ethanolamine	5	2.0	-1.0
	6	1.5	-1.3
	7	1.3	-1.5
Phosphatidyl-serine	5	1.0	-3.2
	6	1.0	-3.2
	7	1.0	-3.2

*defined as the photocurrent with phycocyanin present divided by the photocurrent without phycocyanin

#from (5).

Table 1. The enhancement of the photocurrent by phycocyanin as a function of pH and membrane surface charge.

of the aggregate of phycocyanin involved in the enhancement of the photocurrent.

From the phosphatidyl choline data, we suggest that the concentration of the active aggregate increases as the pH increases. The active aggregate might be the 6S species, which is a trimer (3). The

configuration of the aggregate and the position of the Fe^{+3} ion with respect to the aggregate are unknown.

Portions of the phycocyanin molecule are hydrophobic (3). It was suggested that the effect of the protein on the photocurrent may involve part of the molecule entering the membrane (1). The hydrophilic portion of the molecule is most probably charged. The results using phosphatidyl serine indicate that if the membrane surface charge is large enough, phycocyanin had no effect on the photocurrent.

It is possible that charge-charge repulsions prevent the molecule from entering the membrane and thus no enhancement of the photocurrent is observed.

The results obtained using phosphatidyl ethanolamine are an intermediate case; the membrane surface charge is not large enough to prevent the molecule from entering the membrane. As the pH increases, the concentration of the active aggregate increases. It appears that the charge of the aggregate increases more rapidly, so that the net result is a decrease in the enhancement of the photocurrent.

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REFERENCES

1. Ilani, A. and Berns, D.S. (1972) J. Membrane Biol. 8, 333-356.

2. Berns, D.S. (1975) Abstracts Third Annual Meeting Amer. Society for Photobiol., 104-105.

3. Scott, E. and Berns, D.S. (1965) Biochemistry, 4, 2597-2606.

4. Mangel, M., Ilani, A. and Berns, D.S. (1975) J. Membrane Biol., 171-180.

5. Bangham, A.D. (1968) Prog. Bioph. Mol. Biol., 18, 29-95.