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PROPERTIES OF LIPOSOMES THAT CONTAIN CHLOROPLAST PIGMENTS: PHOTOSENSITIVITY AND EFFICIENCY OF ENERGY CONVERSION

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SUMMARY

Liposomes that contain chlorophyll and carotene are photosensitive. If a gradient of redox potential exists across the liposome membrane, illumination causes charge transport. The quantum efficiency of energy conversion in liposomes is about 0.075. It appears that chlorophyll aggregates are present in the liposomes and that these aggregates are involved in energy conversion.

INTRODUCTION

Bilayer lipid membranes that contain chlorophyll and carotene are known to be photosensitive [1, 2]. When a membrane separated solutions of unequal redox potential, illumination caused charge movement across the membrane. Ilani and Berns [2] measured the quantum efficiency of energy conversion in bilayer lipid membranes. The quantum efficiency is defined as the number of electrons transported divided by the number of photons absorbed. In photosynthetic membranes, the quantum efficiency is about 1 [3, 4]. In bilayer lipid membranes, the quantum efficiency is about 10^{-4} [2].

Chlorophyll aggregates are thought to play an important role in energy conversion in photosynthetic membranes. Ilani and Berns [2] suggested that the organic solvent present in photosensitive bilayer lipid membranes prevents the formation of chlorophyll aggregates. They proposed that the quantum efficiency of energy conversion in bilayer lipid membranes is so low because pigment aggregates were absent [5]. The liposome [6] is a model membrane system that does not contain organic solvent. It was thus conceivable that pigment aggregates exist in liposomes. The following questions were studied.

(1) Are liposomes that contain chlorophyll and carotene photosensitive?

(2) Is the quantum efficiency of energy conversion in liposomes different from that in bilayer lipid membranes?

(3) Are there pigment aggregates in the liposomes?

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MATERIALS AND METHODS

Chlorophyll was purchased from K and K Laboratories in paste form. Egg lecithin was purchased from Sigma Chemicals and β-carotene from Eastman Kodak. The solid materials were dissolved in *tert*-butanol at concentrations of 1–3 mg/ml, 0.1–0.2 mg/ml and 3 mg/ml for chlorophyll, β-carotene and egg lecithin, respectively.

0.5 ml aliquots of each solution were placed in a flask. The flask was connected to a flash evaporator and rotated as the *tert*-butanol evaporated. Once the lipid was dry, a solution of 10^{-4} M $FeCl_3 + 10^{-4}$ M $FeCl_2$ in 0.1 M potassium acetate buffer (pH 5) was added to the flask. Four small glass beads were added, the flask was capped and shaken for 2 or 3 h. The presence of liposomes was ascertained by an osmotic test [6]. After the presence of liposomes was determined, the liposomes were dialyzed against $7 \cdot 10^{-4}$ M KCl in 0.1 M potassium acetate buffer in the dark for 20–24 h. After dialysis, the liposome solutions were transferred to dark bottles and stored at 4 °C. All spectra were obtained using a Cary 14 with 1 cm cuvettes.

The visible absorption spectrum of a preparation of liposomes is shown in Fig. 1. Both chlorophyll peaks were observed. The rapid increase in absorbance as the wavelength decreased was due to scattering of light by the liposomes. The addition of two drops of detergent to each cuvette reduced the spectrum to that of chlorophyll. This indicates that the liposomes were destroyed by the addition of detergent.

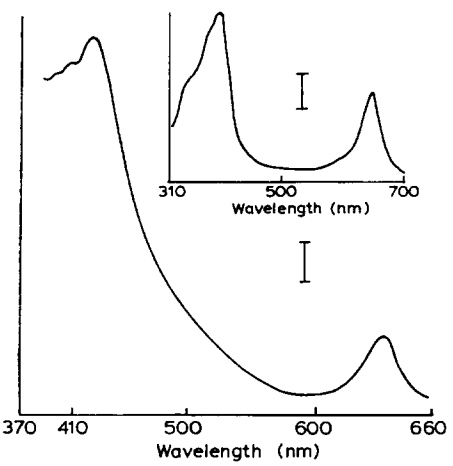

Fig. 1. The optical absorption spectrum of liposomes containing chlorophyll and carotene in the lipid matrix. Liposomes were produced from a mixture of lipid, chlorophyll and carotene. The liposomes contained 0.2 M KCl. The reference for the spectroscopy measurement was 0.2 M KCl. 1 cm cuvettes were used. Inset: optical absorption spectrum of a solution of chlorophyll ($2.4 \cdot 10^{-3}$ mg/ml) in octane. The reference for the spectroscopy measurement was octane. 1 cm cuvettes were used. The bar is 0.1 absorbance unit in each case.

RESULTS

Photosensitivity of the liposomes

The gradient of redox potential was established by mixing a preparation of liposomes with ascorbic acid in 0.1 M potassium acetate buffer (pH 5). Since the

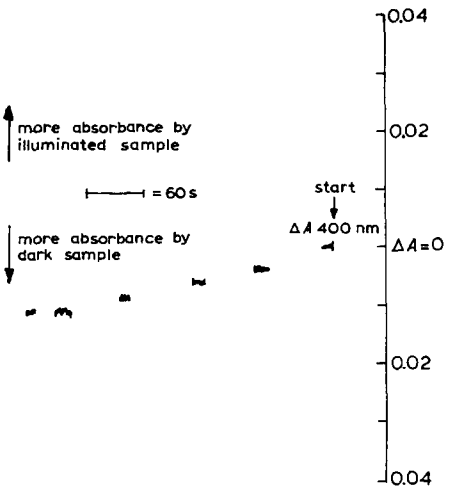

Fig. 2. Measurement of the photosensitive redox reaction in liposomes. Liposomes that contained 10^{-2} M $FeCl_3 + 10^{-2}$ M $FeCl_2 + 10^{-1}$ M potassium acetate buffer (pH 5) were mixed with ascorbic acid so that the final concentration of ascorbic acid was $5 \cdot 10^{-2}$ M. The mixture was divided into two aliquots. One aliquot was kept in the dark and was used as the reference in difference spectroscopy measurements. The other aliquot was illuminated with 660 nm radiation and was used as the sample in difference spectroscopy measurements. Periodically, the difference in absorbance at 400 nm, ΔA, between the aliquot which was illuminated and the aliquot which was kept in the dark was measured. This measurement took about 5 s. The direction of the difference in absorbance indicated more absorption by the aliquot that was kept in the dark.

solution within the liposomes contained $Fe^{3+} + Fe^{2+}$, a redox gradient was established. The solution contained by the liposomes had a greater redox potential than the exterior solution.

A preparation of liposomes was mixed with ascorbic acid so that the ascorbic acid concentration was $5 \cdot 10^{-2}$ M. The mixture was divided into two aliquots. One aliquot was kept in the dark and used as a reference for difference spectroscopy measurements. The other aliquot was illuminated with 660 nm radiation at 1 mW/cm^2 intensity and was used as the sample for difference spectroscopy measurements. At 0.5–1.0 min intervals, the difference in absorbance at 400 nm (ΔA) was measured. The value of ΔA was a measure of the difference in Fe^{3+} concentration. A plot of ΔA against illumination time is shown in Fig. 2. The change in absorbance at 400 nm saturated after finite illumination time. The cause of this phenomenon is unknown. The aliquot that had been kept in the dark had a greater absorbance than the aliquot which had been illuminated. At the end of the experiment, a drop of Triton-X was added to each aliquot. The value of ΔA after the addition of Triton-X was zero. This indicates that the detergent disrupted the liposomes and released the solutions with the liposomes.

Controls

If the Triton-X was added to the liposomes before illumination, the value of ΔA after illumination was zero. A solution of 10^{-2} M $FeCl_3 + 10^{-2}$ M $FeCl_2 +$ 0.1 M potassium acetate buffer (pH 5) was divided into two aliquots. The aliquots

were treated just as the aliquots containing liposomes had been treated. After 10 min of illumination, however, the absorbance at the 400 nm of the sample that had been illuminated was 0.01 absorbance unit greater than the sample that had been kept in the dark. This result indicates an increase in Fe^{3+} concentration upon illumination.

The above experiments indicate that liposomes containing chlorophyll and carotene are photosensitive: if a gradient of redox potential exists across the membrane and the liposomes are illuminated, electron movement from the more reduced side of the membrane to the more oxidized side of the membrane will occur.

In order to compare results from different preparations of liposomes, two terms were defined: (1) ΔA_t; the value of ΔA after t min of illumination. (2) ΔA_T; the value of the total change in absorbance at 400 nm, i.e. in the region of saturation. Then $(\Delta A_t/\Delta A_T)$ is a measure of the rate of the photostimulated redox reactions in the liposomes.

In Fig. 3, the relationship between the rate of the redox reaction and the gradient of redox potential is illustrated. The rate is proportional to the initial slope of the plots of $\Delta A_t/\Delta A_T$ against time. As the gradient of redox potential increased, the rate of the photosensitive redox reaction increased. This result is similar to the observation by Ilani and Berns [2] that the photovoltage response in bilayer lipid membranes increased as the gradient of redox potential increased.

In Table I, the relationship between intensity of illumination and the value of ΔA is reported. As the intensity of illumination increased, the difference in absorbance at 400 nm increased. This result is also similar to a corresponding observation with bilayer lipid membranes [2].

We conclude that liposomes that contain chlorophyll and carotene are photosensitive and that some of the properties of the liposomes are similar to those of photosensitive bilayer lipid membranes.

A possible difference in the properties of the two kinds of membrane, as

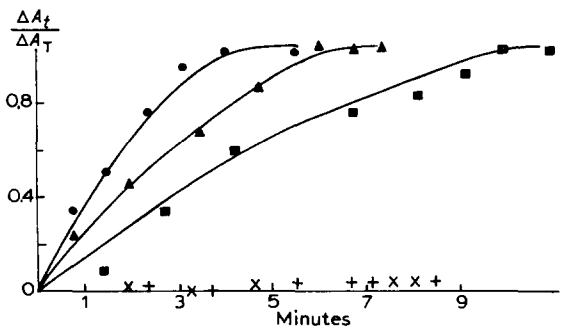

Fig. 3. The rate of photosensitive redox reactions in liposomes as a function of ascorbic acid concentration. The liposomes contained 10^{-2} M $FeCl_3 + 10^{-2}$ M $FeCl_2 + 10^{-1}$ M potassium acetate buffer (pH 5). The liposomes were mixed with solutions of ascorbic acid + buffer so that the concentration of ascorbic acid was 10^{-1} M (●), $5 \cdot 10^{-2}$ M (▲) or $2.5 \cdot 10^{-2}$ M (■). $\Delta A_T = 0.012$ (●), 0.012 (▲) and 0.011 (■). Other details as in Fig. 2. Two experiments in which chlorophyll was absent from the liposomes are indicated by (+). Experiments in which carotene was absent from the liposomes are indicated by (×). In these experiments, the concentration of ascorbic acid was 10^{-1} M. Other details as in Fig. 2.

TABLE I

INTENSITY OF ILLUMINATION AND THE PHOTORESPONSE OF PHOTOSENSITIVE LIPOSOMES

The incident intensity of illumination was modified by placing neutral density filters in the light path [2]. A mixture of liposomes $+5 \cdot 10^{-2}$ M ascorbic acid was illuminated for 3 min with 660 nm radiation. A mixture of identical composition was kept in the dark. After 3 min of illumination at 660 nm, the difference in absorbance at 400 nm was measured.

Relative intensity of illumination	ΔA after 3 min
1.0	0.0015
2.3	0.0040
3.3	0.0060
4.7	0.0090
6.6	0.0100

mentioned in the introduction, could be the value of the quantum efficiency of energy conversion. Consequently, the quantum efficiency was determined.

Measurements of the efficiency of energy conversion

The quantum efficiency of energy conversion was defined as the number of electrons transported divided by the number of photons absorbed. The number of electrons transported was the same as the number of Fe^{3+} ions reduced, which could be calculated from the change in absorbance at 400 nm. The number of Fe^{3+} ions reduced was

$$\Delta N = \frac{\Delta A}{\varepsilon} \cdot V \cdot N_A \tag{1}$$

where ΔN is the number of Fe^{3+} ions reduced, ΔA is the change in absorbance at 400 nm, ε is the molar extinction coefficient of Fe^{3+} at 400 nm, V is the sample volume and N_A is Avogadro's number. For the experiments reported here, we have

$$\Delta N = (\Delta A)\,(1.8 \cdot 10^{18}) \tag{2}$$

The amount of light absorbed by the sample was

$$\Delta I = (I_i - I_t)\Delta t \tag{3}$$

where I_i, I_t are the incident and transmitted intensities, respectively, and Δt is the time of illumination. If α is the absorbance of the liposome preparation at 660 nm, we also have that

$$\alpha = \log(I_i/I_t) \tag{4}$$

$$\alpha = \log(I_i/(I_i - \Delta I)) \tag{5}$$

When Eqn. 5 is solved for ΔI, we obtain

$$\Delta I = I_i(1 - 10^{-\alpha}) \tag{6}$$

A 1 mW/cm^2 intensity of incident radiation at 660 nm corresponds to $1.7 \cdot 10^{15}$ photons/s. The number of photons absorbed by the sample was

$$\Delta P = 1.7 \cdot 10^{15}(1-10^{-\alpha})\Delta t \tag{7}$$

Thus,

$$\text{quantum efficiency} = \frac{\Delta N}{\Delta P} = \frac{\Delta A(1.06 \cdot 10^3)}{\Delta t(1-10^{-\alpha})} \tag{8}$$

Calculations of the quantum efficiency were performed for twenty different preparations of liposomes. The result was quantum efficiency $= 0.075 \pm 0.015$ (r.m.s.). The quantum efficiency of liposomes was much higher than that of bilayer lipid membranes, which was less than 10^{-4} [2]. Ilani and Berns [5] suggested that the quantum efficiency might be improved by the presence of pigment aggregates. Thus, it was possible that the liposomes contained pigment aggregates.

Chlorophyll aggregates in bilayer lipid membranes and liposomes

When chlorophyll aggregates are present, a long wavelength ($\lambda \geqslant 700$ nm) absorption band is observed [7]. No long wavelength absorption bands were observed in the absorption spectra of solutions used to form photosensitive bilayer lipid membranes. This result is consistent with the conclusion that chlorophyll aggregates are not present in bilayer lipid membranes [2, 5].

Some long wavelength spectra of liposomes preparations are shown in Fig. 4. The "opal glass technique" [8] was used to minimize scattering. In each liposome preparation, an absorption band was observed around 750 nm. It appears that some

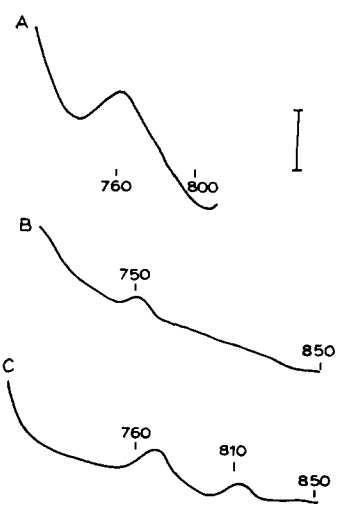

Fig. 4. Long wavelength absorption spectra of liposome preparations. Liposomes which contained 10^{-2} M $FeCl_3 + 10^{-2}$ M $FeCl_2 + 10^{-1}$ M potassium acetate buffer (pH 5) were used as the sample. A solution of 10^{-2} M $FeCl_3 + 10^{-2}$ M $FeCl_2 + 10^{-1}$ M potassium acetate buffer (pH 5) was used as the reference. All measurements correspond to 1 cm cuvettes. The "opal glass technique" was used to minimize the effects of scattering. Absorption in the 720–760 nm range corresponds to a molecular aggregate of chlorophyll [7]. The bar corresponds to 0.01 absorbance units in A and C and to 0.1 absorbance unit in B.

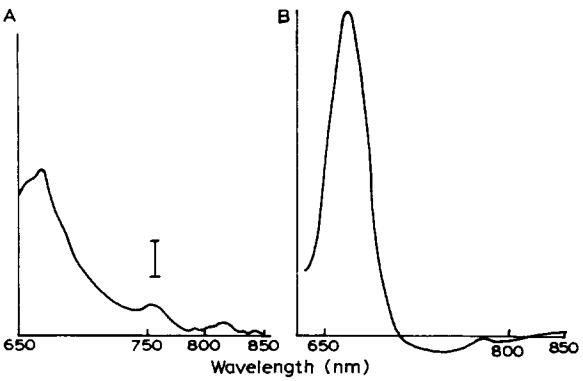

Fig. 5. Disruption of the liposomes and disappearance of long wavelength absorbances. (A) The optical absorption spectrum of a preparation of liposomes. Other details as in Fig. 4. (B) The optical absorption spectrum of the same preparation of liposomes after the addition of two drops of Triton-X. The addition of Triton-X destroyed the liposomes. After the addition of Triton-X, the long wavelength absorption band disappeared. The bar corresponds to 0.01 absorbance unit.

of the chlorophyll in the liposomes was present in a molecular aggregate.

In Fig. 5 the absorption spectra of a preparation of liposomes before and after the addition of detergent are shown. The addition of detergent led to (1) the disappearance of the absorption band centered around 750 nm and (2) an increase in the red absorption peak, from 0.04 to 0.09 absorbance units. In addition, the position of the red peak shifted from 663 to 658 nm. These results indicate that some of the chlorophyll in the liposomes was in the form of a molecular aggregate. It is not clear, however, if the aggregate was responsible for the relatively high quantum efficiency of energy conversion.

TABLE II

THE EFFECT OF COMPOUNDS THAT DISRUPT CHLOROPHYLL AGGREGATES ON THE QUANTUM EFFICIENCY OF ENERGY CONVERSION IN LIPOSOMES

A preparation of liposomes+ascorbic acid was divided into six aliquots. In the first experiment, the experimental procedure discussed in Fig. 2 was used without modification. In the second experiment, pyridine (1 %, v/v) was added to each aliquot of liposomes+ascorbic acid before the beginning of the illumination with 660 nm radiation. In the third experiment, the temperature of each aliquot was raised to 50 °C and maintained at 50 °C during illumination with 660 nm radiation. In all three experiments, the difference in absorbance at 400 nm after 7.5 min of illumination with 660 nm radiation was recorded. The difference in absorbance at 400 nm was a measure of the efficiency of energy conversion (Eqn. 8). The modification of the liposomes by pyridine or high temperature lead to a substantial fall in the quantum efficiency of energy conversion.

Modification of the liposomes	Relative quantum efficiency
None	1.00
1 % pyridine	0.18
50 °C	0.24

Involvement of aggregates in energy conversion

If the chlorophyll aggregates that were present in the liposomes were involved in energy conversion, disruption of the aggregates would decrease the efficiency of energy conversion. Pyridine [9] and high temperature [10] are thought to disrupt chlorophyll aggregates. In Table II, the results of adding pyridine and raising the temperature of a preparation of liposomes are shown. The quantum efficiency of energy conversion was decreased by more than 75 % by either modification.

The addition of pyridine or increase of temperature to 50 °C eliminated the long wavelength chlorophyll absorption band but did not affect the 660 nm band. Elimination of the long wavelength band and observation of a photosensitive redox reaction indicate that the chlorophyll aggregates, but not the liposomes, were destroyed. That the quantum efficiency of energy conversion was less after modification than before suggests that the aggregates are partially responsible for the relatively high quantum efficiency of energy conversion in liposomes.

CONCLUSION

The three questions posed at the beginning of this paper have been answered. Liposomes that contain chlorophyll and carotene are photosensitive. Some of the properties of photosensitive bilayer lipid membranes and photosensitive liposomes are similar. The efficiency of energy conversion in liposomes is much greater than the efficiency in bilayer lipid membranes. It appears that the chlorophyll aggregates present in the liposomes are partially responsible for the higher efficiency of energy conversion. The mechanism involved in energy conversion in liposomes of bilayer lipid membranes is still unknown. It appears that pigment aggregates may play an important role in the conversion of photonic energy to electronic energy.

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