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PHOTOINDUCED ELECTRON TRANSPORT ACROSS LIPOSOMAL MEMBRANE USING CHLOROPHYLLIN A

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Photoinduced electron transport across the bilayer lipid membrane of liposome was studied by using chlorophyllin *a*, a water-soluble chlorophyll derivative, without an electron carrier. The reduction of ferricyanide by sodium ascorbate was observed on irradiation with visible light under anaerobic condition.

The membrane-like structure plays the important role in the bioenergetic system. It is well-known that thylakoid membrane in chloroplast is indispensable to photosynthesis.

Though there have been many reports on chlorophyll-sensitized electron transfer reaction in liposome as a model of photosynthesis, little has been reported about photosensitized electron transport across liposomal membrane. Mangel and co-workers demonstrated that photosensitized reduction of Fe^{3+} inside the liposome by ascorbate outside could take place only in the presence of both chlorophyll and β-carotene.[1)] From the results, they concluded that chlorophyll acts as a photosensitizer, while β-carotene plays a role of an electron carrier.

In another investigation,[2,3)] the reduction of Cu^{2+} or ferricyanide in the outer phase by ascorbate within the vesicle was observed on irradiation. Contrary to the results of the experiments described above, it was shown that the reduction rate didn't change significantly if β-carotene was also incorporated into liposomes. The reasons for this discrepancy are not clear.

We think that if the photosensitizer does not possess any long alkyl chains, the pigment can move in the phospholipid phase and electron transport can occur in the simpler system without using any carotenes and quinones. In the previous papers,[4,5,6,7)] we demonstrated that electron transport model sensitized by the dyes obtained commercially. The dyes are methylene blue, new methylene blue, and phenosafranine which have no long alkyl chains.

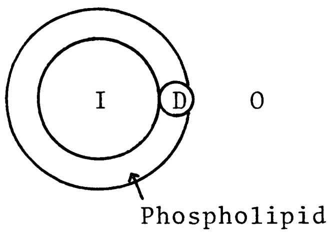
In this paper, we investigated that photoinduced electron transport across the lipid membrane of liposomes using chlorophyllin *a*. Chlorophyllin is a water-soluble derivative of chlorophyll, the product of saponification of chlorophyll and a fairy green pigment which resembles chlorophyll in its photochemical reactivity.[8,9,10)]

Chlorophyll *a* (Chl) was extracted from fresh spinach leaves with acetone and precipitated by distilled water and dioxane,[11] followed by chromatographic procedure using DEAE-Sepharose CL-6B and Sepharose CL-6B columns.[12] Chlorophyllin *a* (Chln) was prepared according to the procedure of Oster *et al.*.[13]

The experiments were carried out in the liposome system (Fig. 1), which was prepared by the following way.

Egg-yolk lecithin was freeze-dried. When liposomes containing Chl were prepared (System D), a desired amount of Chl dissolved in benzene was added to lecithin solution and co-lyophilized. Then it was dispersed in an aqueous solution of 1 M (1 M=1 mol dm^{-3}) potassium ferricyanide (FCN) buffered by 1 M Tris-Cl, 0.1 M KCl (pH 7.5) by sonication. Untrapped FCN was removed by gel-filtration over a column of Sephadex G-50. When Chln was used in place of Chl (Systen C), Chln in the buffer solution was added to the liposome dispersion obtained by gel-filtration and incubated. After the mixture was degassed by passing argon gas, the solution of sodium ascorbate (AscNa) was added.

The absorption spectra of Chln in the buffer solution (1) and in the liposome system (System A) encapsulating only the buffer solution (2) are shown in Fig. 2. The spectrum in an aqueous solution has two peaks at 418 nm and 640 nm. But in the spectrum in the liposome system, the long-wavelength peak shifted to 660 nm. The shift was also observed when a water-soluble polymer, polyvinylpyrrolidone was added to the solution of Chln.[13,14] Such a property of the spectrum in the liposome system in the present work is similar to that in the liposome system



System A
I=Buffer, O=Buffer
D=Chln

System B
I=FCN, O=Buffer
D=Chln

System C
I=FCN, O=AscNa
D=Chln

System D
I=FCN, O=AscNa
D=Chl

Fig. 1. Various liposome systems.

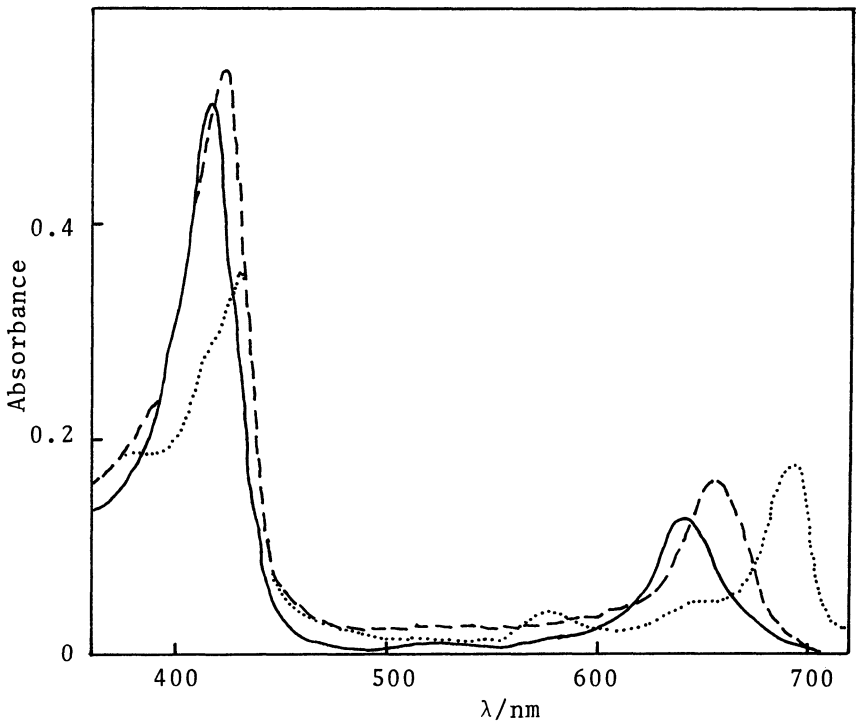
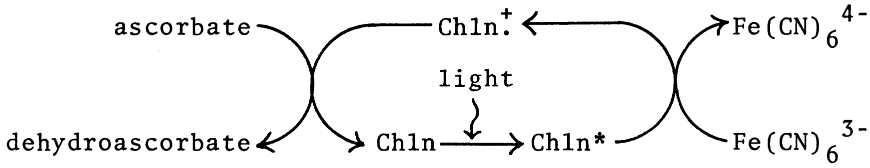


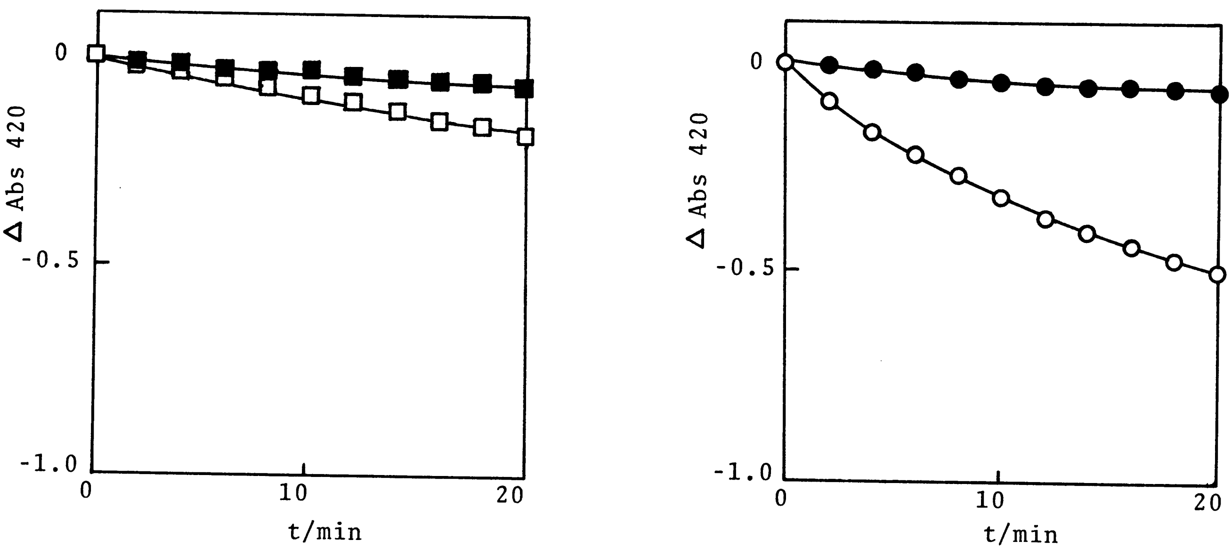
Fig. 2. Absorption spectra of chlorophyllin *a* in the various systems. (1):in the buffer solution (——) (2):in the liposome system (A) (---), (3):in the liposome system (B) (·······)

containing Chl.[2,3] This shift was thought to be derived from the aggregation of Chln. The absorption spectrum of Chln added to the liposome system (System B) encapsulating FCN (3) is also shown in Fig. 2. The long-wavelength peak at 660 nm shifted to 690 nm. This shift was also observed when Chln solution was added to the aqueous solution of FCN. Such results indicate that Chln added to the liposome system was incorporated into the lipid phase of liposomes and existed in the inner surface of the lipid phase. The shift from 660 nm to 690 nm was thought to be derived from some interaction of the central Mg of Chln and FCN, but in detail, we cannot explain it.

Fig. 3 shows the decrease of the absorbance at 420 nm with time of irradiation. Light was irradiated with 500 W Xenon lamp and glass filters were used to isolate red light. The decrease of the absorbance at 420 nm was attributed to the reduction of FCN because change in the absorption of Chl or Chln was negligibly small. The reduction of FCN was not observed without irradiation even in the presence of AscNa. The reduction was observed only on irradiation in the presence of AscNa in the outer aqueous solution. The rate of FCN reduction using Chln was much higher than that using Chl. The rate using Chl did not increase by the high concentration of Chl. This result was thought as follows.

Chln, which is different from Chl, has no long alkyl chain (phytol). So, Chln incorporated into the membrane has more mobility than Chl and can move between the outer surface and the inner surface of the membrane. And the carboxyl groups may be able to transport proton because Chln is a tri-carboxylic acid. Electron transport from AscNa to FCN is thought to occur in this mechanism.





(a) [Chl]=5.0 μM, [AscNa]=10 mM
□:irradiated ■:non-irradiated

(b) [Chln]=3.0 μM, [AscNa]=10 mM
○:irradiated ●:non-irradiated

Fig. 3. Change of absorbance at 420 nm with time of irradiation.

In the present study, we demonstrated that chlorophyllin *a* is a useful pigment for the simulation of photosynthesis.

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