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## Laser Flash Photolysis Studies of Chlorophyll-Copolyptide Complexes in Aqueous Surfactant Solutions

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### Abstract

Photoinduced electron transfer between tyrosine-containing copolyptide and chlorophyll a (Chl) in aqueous surfactant solutions was studied with laser flash photolysis spectroscopy. The result indicated that the triplet state of Chl participates in the photoreaction of the present systems and that conformation-dependent photoinduced electron transfer effectively occurs from tyrosine to Chl in the Chl-copolyptide complexes formed through hydrophobic interaction.

### Introduction

In photosynthetic materials, the physical properties of chlorophyll molecules organized in pigment-protein complexes are necessarily influenced by the interaction with the surrounding protein matrix (1). Electron transfer involving remote sites on proteins and peptides has been the theme of many recent studies in photochemical and biophysical investigations. It has been pointed out that the conformation of the matrix and that of the complex as a whole (the orientation of chlorophyll and its related compounds relative to the protein in particular) are of considerable importance for light-induced electron transfer in photosynthetic reaction centers (2). However, the extent, magnitude and mechanism of this influence is not completely understood. For the purpose of investigating electron transfer between a photoexcited chromophore and the side chain of an electroactive amino acid, we have so far prepared an aqueous model system, consisting of chlorophyll a (Chl) incorporated into copolyptide-surfactant complexes formed by a cooperative binding and have investigated spectroscopic properties and

photoinduced energy- and electron-transfer processes of Chl-copolyptide complexes in aqueous surfactant solutions (3-7).

For example, Kusumoto and Kurawaki (3) have investigated spectroscopic properties of Chl-copolyptide carrying glutamate and tyrosine (GT)-cetyltrimethylammonium chloride (CTAC) systems, and indicated that Chl molecules are incorporated into micelle-like clusters formed by a cooperative binding of an CTAC ion to a negatively charged GT ion to give rise to Chl-GT complexes and efficient energy transfer from tyrosine to Chl in the Chl-GT complexes takes place with an increase in CTAC concentration. In addition, we have also carried out spectroscopic and potentiometric studies of Chl-copolyptide consisting of ornithine and tyrosine (OT) complexes in aqueous sodium dodecyl sulfate (SDS) solution; we could characterize Chl-OT complexes in the presence of SDS by correlating various spectroscopic data with the degree of binding of surfactant to copolyptide in aqueous solution (4,5) and indicate that photoinduced electron transfer in  $\alpha$ -helical copolyptides carrying aromatic amino acids occurs by through-space mecha-

nism (7).

In this paper, we studied photoinduced electron transfer between Chl and tyrosine-containing copolypeptide in aqueous surfactant solutions with laser flash photolysis method and assigned new transient species occurring in the photoreaction on flash excitation of Chl-copolypeptide-surfactant systems based on the result of electrochemical study.

## Experimental

### Materials

Chl (> 99.8%) was purchased from Wako Pure Chemical Industries Ltd. Tyrosine-containing copolypeptides such as GT and OT used in this work were obtained from Sigma Chemicals Co.; their molar ratio was 4:1 and molecular weights were 80000 and 23000, respectively. SDS (protein research grade of Nakarai Chemicals) and CTAC (GR grade of Nakarai Chemicals) used as surfactants, were purified by recrystallization three times from ethanol. The concentration of copolypeptide, based on the mean residue concentration of tyrosine, and that of Chl were fixed at  $1.0 \times 10^{-4}$  and  $1.0 \times 10^{-5}$  M ( $1M = 1 \text{ mol dm}^{-3}$ ), respectively, through the experiment. Laboratory deionized water was distilled twice. The pH of the sample solutions was not controlled but was found to be about 6.5.

### Measurements

Absorption and circular dichroism (CD) spectra were measured with a Shimadzu MPS-2000 spectrophotometer and a JASCO J-720 spectropolarimeter, respectively. The detail optical system of laser flash photolysis apparatus has been described in a previous paper (6). In brief, transient absorption spectra and decay curves were measured by using a  $\text{Nd}^{3+}$ : YAG laser (Spectron Laser Systems Ltd., model SL402) and a monitoring Xe-lamp systems; the third harmonic (355 nm) of this laser was used as the excitation light for exciting Chl. The average laser power at the sample point was less than 10 mJ / pulse to avoid physical damage to the sample cell and possible biphotonic or other nonlinear processes in the sample solutions. The photons were detected with an RCA 8850 photomultiplier tube equipped

with a thermoelectric cooler. The signal from the photomultiplier tube was stored and analyzed by using a digital storage oscilloscope (Tektronix, model 2224). The delay time after the excitation was controlled by the use of a digital delay / pulse generator (Stanford Research, model DG535). The sample solution in a quartz cell with graded seals was deaerated by means of repetitive freeze-pump-thaw cycles. Controlled-potential electrogeneration of cation and anion radical species of Chl was performed at the platinum electrode with a Hokuto Denko HA-301 potentiostat (8). Tetrabutylammonium perchlorate (TBAP) was used as a supporting electrolyte. All measurements were made at room temperature.

## Results and Discussion

### Transient absorption spectra

Typical transient absorption spectra of Chl-GT system in aqueous CTAC solution are shown in Fig. 1. The transient spectrum measured at 10 ns after the excitation for Chl-CTAC system (Fig. 1a) has a negative absorption band at 440 nm and a positive broad one at 465 nm; this spectrum corresponds to triplet-triplet absorption spectrum of Chl in aqueous solution (6,9). The negative and strong absorption in the spectrum can be undoubtedly ascribed to the Soret-band bleaching of Chl in the ground state. The transient decay curve monitored at 465 nm exhibited single-exponential decay with a lifetime of 200  $\mu$ s, indicating that this absorption component around 465 nm can be ascribed to the triplet state of Chl ( $^3\text{Chl}$ ).

Upon addition of GT to the Chl-CTAC system, the transient absorption spectrum (Fig. 1b) shows two interesting absorption bands at 390 and 520 nm following the quenching of the 465-nm band assignable to  $^3\text{Chl}$ . In this system, Chl molecules are incorporated into  $\alpha$ -helical GT-CTAC complexes formed by a cooperative binding of a CTAC ion to a negatively charged GT ion to give rise to Chl-GT complexes (3,7). This transient spectrum was almost similar to that obtained for Chl-CTAC system in the presence of GT with a molar ratio of 1:1 (6,7), as shown in Fig. 1c. This fact, therefore, suggests the

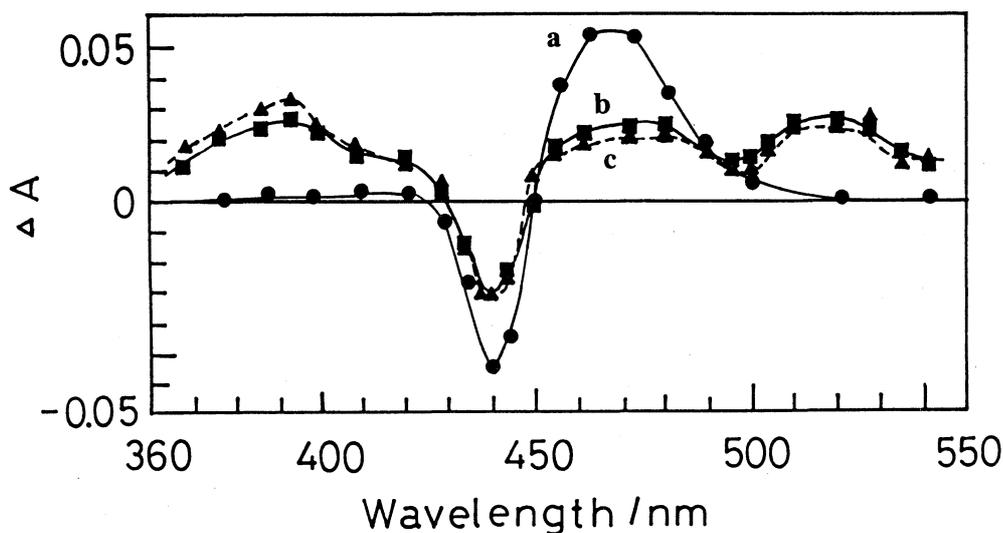


Fig. 1. Transient absorption spectra of (a) Chl-CTAC, (b) Chl-GT-CTAC and (c) Chl-GT (molar ratio 1:1)-CTAC systems. [CTAC] = 0.25 mM.

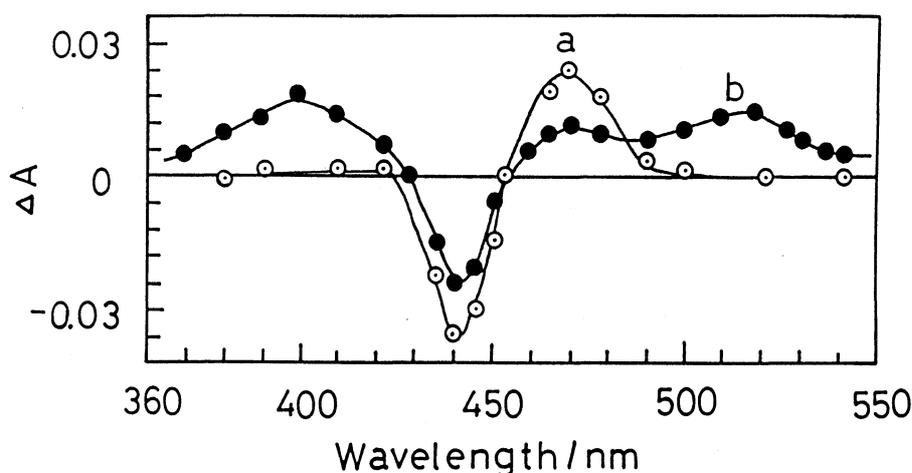


Fig. 2. Transient absorption spectra of (a) Chl-SDS and (b) Chl-OT-SDS systems. [SDS] = 0.20 mM.

formation of other intermediates occurring in photoreaction on flash excitation of Chl-GT complexes, in addition to  $^3\text{Chl}$ . Transient decay profiles measured at 500-530 nm were fitted to first-order kinetics and the lifetime of this absorption component was determined to be 820  $\mu\text{s}$ . On the other hand, the decay curves monitored at 460-480 nm exhibited double-exponential decay with lifetimes of 190 and 700  $\mu\text{s}$ , indicating the coexistence of a short-lived and a long-lived components. This short-lived component can be assigned to  $^3\text{Chl}$ , judging

from the similarity of absorption-peak wavelength and the lifetime with those obtained for  $^3\text{Chl}$  shown in Fig. 1a. A long-lived component may be some transient radical species such as cation or anion radical of Chl or tyrosine which results from electron transfer between Chl and tyrosine-containing copolyptide in the present systems. We suggest, therefore, that the  $^3\text{Chl}$  participates in the photoreaction of the present system and that the quenching of  $^3\text{Chl}$  with GT may be due to electron transfer.

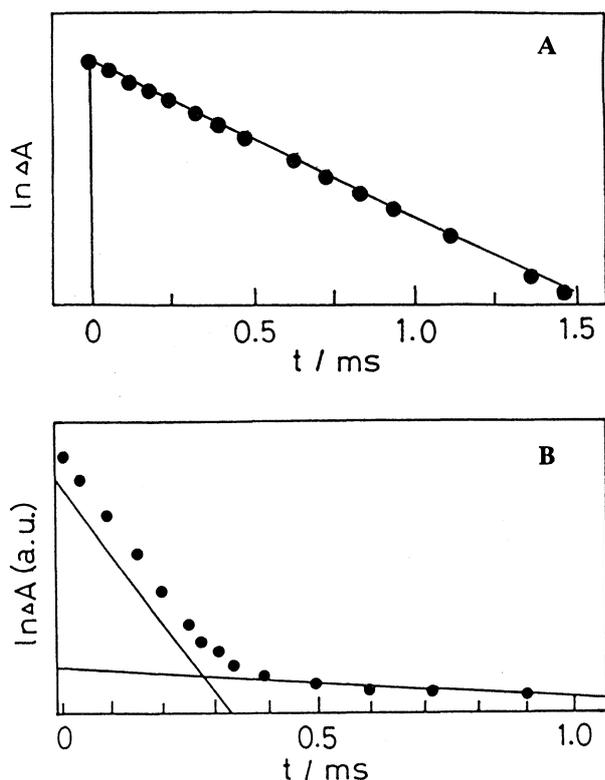


Fig. 3. Transient decay curves monitored at (A) 520 nm and (B) 470 nm for Chl-OT-SDS system. [SDS]=0.20 mM.

The transient absorption spectra of Chl-SDS system in the absence and presence of OT are shown in Fig. 2. Upon addition of OT to Chl-SDS system, the 470-nm absorption band assignable to  $^3\text{Chl}$  is effectively quenched and two positive absorption bands at 400 and 520 nm appear in the spectrum (Fig. 2b). The transient decay curves monitored at 520 and 470 nm are shown in Fig. 3. The decay curve at 520 nm exhibits single-exponential decay with a lifetime of  $810 \mu\text{s}$  and that monitored at 400 nm also presents the same lifetime (Fig. 3A), while that monitored at 470 nm exhibits double-exponential decay with lifetimes of 190 and  $700 \mu\text{s}$  (Fig. 3B). The absorption peaks and the decay times of transient species obtained for Chl-OT system are almost the same with those of the transient species found in Chl-GT-CTAC systems shown in Fig. 1. Therefore, it can be concluded that  $^3\text{Chl}$  participates in the photoreaction of Chl-copolypeptide complexes formed via hydrophobic interaction and that photoinduced electron transfer between Chl and tyrosine-containing copolypeptide may take place

following the formation of cation or anion radical species of Chl or tyrosine.

#### Assignment of transient species

In order to assign new transient species produced after flash excitation of Chl-copolypeptide complexes, we measured transient absorption spectra of Chl-CTAC system in the presence of ascorbic acid which acts as an electron donor and absorption spectra of electrochemically generated cation and anion radical species of Chl in aqueous solution containing a supporting electrolyte. As shown in Fig. 4, flash excitation of Chl-CTAC system in the presence of ascorbic acid gives rise to essential changes of Chl absorption spectra in the visible region; transient absorption bands with a lifetime of  $820 \mu\text{s}$  can be observed at 395 and 520 nm (Fig. 4B). Chibisov (10) has investigated the photochemical reactions of Chl intermediates in the presence of ascorbic acid by flash photolysis method and indicated the direct evidence of  $^3\text{Chl}$  participation in photoreduction process resulting in the formation of anion radical of Chl ( $\text{Chl}^-$ ). The transient spectra in Fig. 4A were almost similar to those obtained by Chibisov. Thus, the absorption at 390-400 and 510-530 nm observed for Chl-copolypeptide systems can be assigned to  $\text{Chl}^-$  which results from electron transfer from tyrosine to Chl.

Absorption spectra of electrochemically generated cation radical of Chl ( $\text{Chl}^+$ ) and  $\text{Chl}^-$  are shown in Fig. 5. The absorption peaks of  $\text{Chl}^+$  produced at a final stage of electrolysis at 0.65 V in aqueous solution are observed at 416, 540, 667 and 805 nm, while those of  $\text{Chl}^-$  produced at a final stage of electrolysis at  $-0.85 \text{ V}$  are found at 395, 520, 667 and 800 nm. The absorption maxima of the transient species observed for Chl-copolypeptide-surfactant systems (Figs. 1 and 2) are almost similar to those of electrochemically generated  $\text{Chl}^-$ , except for the difference of relative absorbance.

In addition, the absorption spectra of electrochemically generated cation radical of tyrosine observed for tyrosine-containing copolypeptides in aqueous solution showed an absorption peak around 470 nm (data not shown). Therefore, the

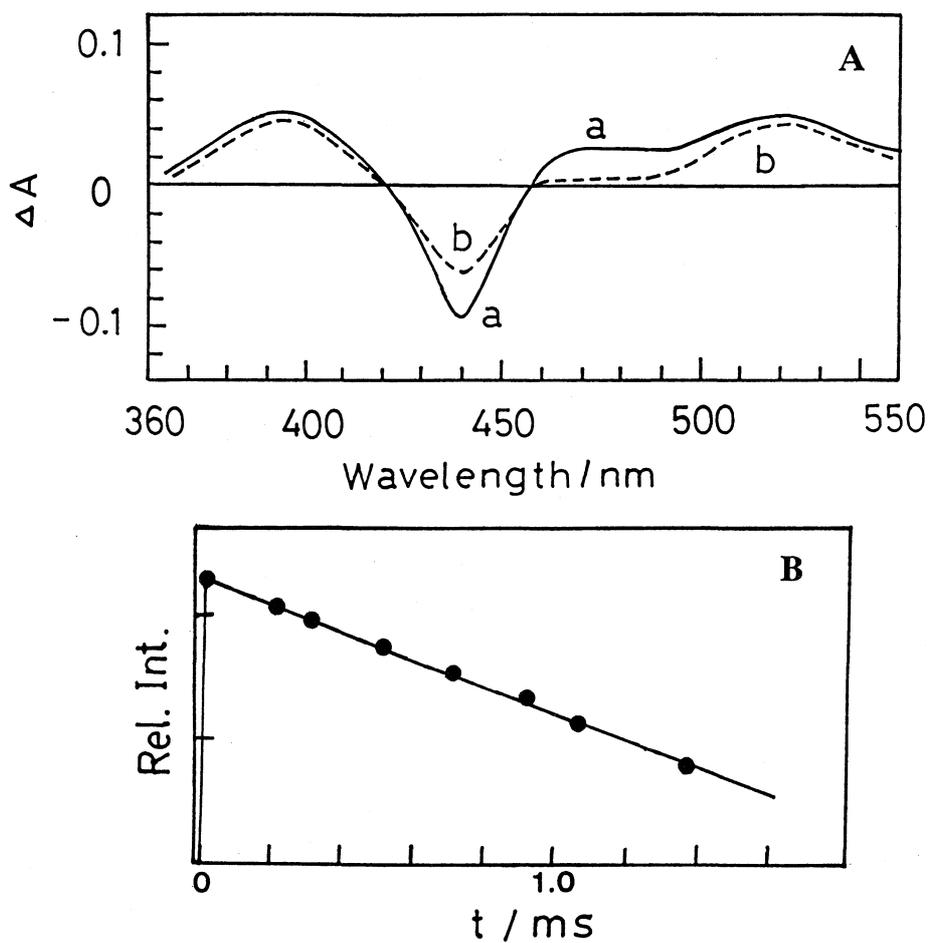


Fig. 4. Transient absorption spectra (A) and decay curve monitored at 520 nm (B) of Chl in the presence of ascorbic acid. [ascorbic acid] = 0.1 mM.

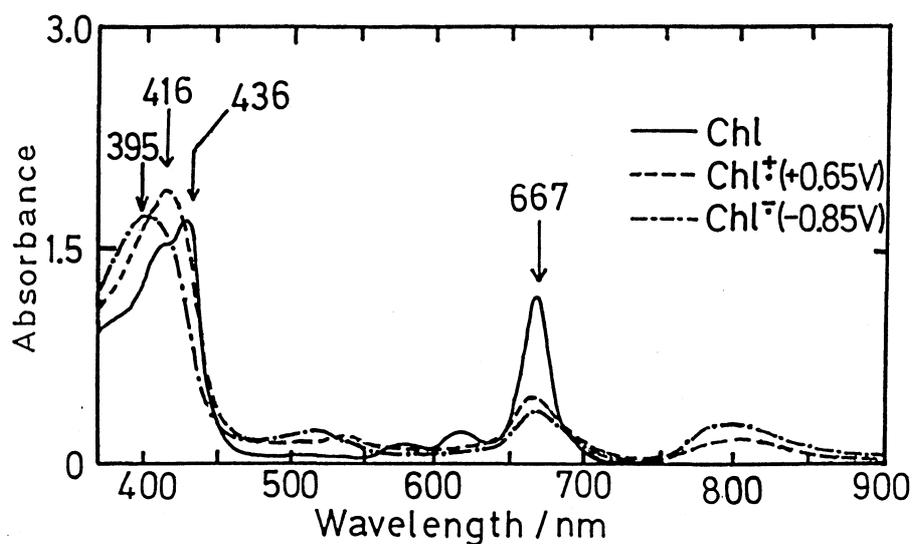


Fig. 5. Absorption spectra of neutral Chl (—), Chl<sup>+</sup> (---) and Chl<sup>-</sup> (-.-) produced at a final stage of electrolysis in aqueous solution containing 0.1 M TBAP.

470-nm absorption band with the lifetime of 700  $\mu$  s can be assigned to the cation radical of tyrosine.

Judging from the above results, it can be concluded that  $^3\text{Chl}$  participates in the photoreaction of the present system and that photoinduced electron transfer effectively occurs from tyrosine to Chl in the Chl-copolyptide complexes formed through hydrophobic interaction (6,7). These results indicate that the lowest  $^3\text{Chl}$  is located at a sufficiently low energy level to be able to accept an electron from tyrosine which acts as a reductant, resulting in the formation of  $\text{Chl}^-$  and the cation radical of tyrosine. Vibrational studies of  $\text{Chl}^-$  produced on flash excitation of the present systems are now in progress (11).

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