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The Light-driven Electron Transfer Reaction of Chlorophyll a–Lumiflavin in Micelle

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The light-driven electron transfer reaction for the system of chlorophyll a–lumiflavin in micelles was studied by means of flash photolysis. In the sodium dodecyl sulfate micellar solution, the chlorophyll a cation radical appeared on the flash excitation and then decayed by means of a second-order process under aerated conditions as well as under deaerated conditions (the radical was not observed in dioxane). The decay parameter in the aerated solution was smaller than that in the deaerated solution by a factor of twenty. The difference in the decay parameter was explained in terms of the function of the surface charge of micelles. In the deaerated solution, the chlorophyll a cation radical decays by means of a reaction with lumiflavin semiquinone of no charge; in the aerated solution, however, it decays by means of a reaction with the O_2^- produced by the electron transfer from lumiflavin semiquinone to oxygen. The latter decay reaction is retarded by the charge on the micelles.

In the photosynthesis, it is established that the electron is transferred from the excited chlorophyll a (Chl a) in P700 (the primary reaction center of the photosystem I of chloroplasts) to $NADP^+$-reductase, which has flavin adenine dinucleotide (FAD) as the prosthetic group, through ferredoxin (iron–sulfer protein). Wang *et al.* studied the complexes of Chl a derivatives, with flavins as model complexes for P700, and observed the light-induced electron-transfer reactions from the chlorophyll to the flavin.[1] The electron-transfer reactions from Chl a to *p*-benzoquinone[2] or to Chl a[3,4] were also examined under suitable homogeneous conditions, and it was concluded that the electron transfer proceeded from the singlet excited state of Chl a[3] or from the triplet state.[2,4]

In the chloroplast membrane, the specific environmetal effect must play an important role. Micellar systems have been examined[5] as models for membrane, especially in order to clarify the interfacial effects. In this work, kinetical studies have been made of Chl a–lumiflavin in aqueous micellar systems. Photolyses under different concentrations of dissolved oxygen were performed. Lumiflavin was used instead of riboflavin as an electron acceptor to avoid the interference of the reactive side chain, as in our previous studies of the sensitized photooxygenation.[6] Photolysis in dioxane was also examined in order to make a comparison with that in an ordinary solution.

Experimental

Materials. The chlorophyll a was extracted from spinach, purified chromatographically by the procedure of Strain and Svec,[7] and stored at −20 °C. The lumiflavin was synthesized as has been described by Hemmerich *et al.*[8] The sodium dodecyl sulfate (SDS) was recrystallized from ethanol, and the hexadecyltrimethylammonium bromide (CTAB) was recrystallized from ethanol–ether. The dioxane was freshly distilled after several days of refluxing over sodium.

Procedure. All the sample solutions were prepared just before their use. The Chl a–SDS solutions were prepared by the following procedure. The Chl a was first dissolved in ethanol, mixed with a concentrated SDS solution containing a buffer, and finally diluted with the buffer solution. The final solutions contained 2% ethanol by volume. In anionic micellar solutions such as SDS solutions, the local pH value around the micelles is lower than the bulk according to the electrostatic potential of the micellar surface. Therefore, the SDS solutions were required to be rather basic (pH 9.0) to avoid the elimination of Mg from Chl a. As the buffer, *N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS, 0.05 M†) was used. The concentration of Chl a was adjusted to be not less than 7 μM, because it is less stable at lower concentrations. Chlorophyll a dissolved in this manner was stable for two hours, judging from the absorption spectrum.

The Chl a–CTAB solutions were prepared just as the Chl a–SDS solutions, but the solutions were adjusted to pH 7.2 with a sodium phosphate buffer. Unlike anionic micellar solutions, the local concentration of the hydroxide ion around the CTAB micelles is relatively high, and it tends to cause the allomerization (oxidative degradation) of Chl a.

The critical micelle concentration (CMC) values of the surfactants were determined by the use of a stalagmometer. The amounts of dissolved oxygen in aerated solutions were measured by means of Winkler's method.[9] The solutions with reduced concentrations of oxygen were prepared by purging with mixtures of nitrogen and oxygen of known compositions.

Flash photolysis was carried out in a 1-cm rectangular quartz cell or in a 10-cm cylindrical quartz cell, with exciting light from xenon flash lamps (9 μs half-maximum duration) through red glass filters (cut off at 580 nm) to avoid the excitation of lumiflavin. The fluorescence was measured with a Hitachi MPF-2A fluorescence spectrophotometer.

Partition of Reactants between SDS Micellar and Water Phases. In the SDS micellar solution, Chl a is exclusively present in the micellar phase, because Chl a is insoluble in water. On the other hand, lumiflavin is present in both micellar and water phases. The partition of lumiflavin into them was determined from the measurements of the change in the fluorescence intensity of lumiflavin with different concentrations of SDS. The fluorescence intensity ratio of micellar solutions to the solution without micelles was independent of the lumiflavin concentration in the range from 30 μM to 100 μM (the solubility limit in the bulk). Therefore, the distribution of lumiflavin can be treated as the partition equilibrium.

Putting the fluorescence yields in aqueous and micellar phases as ϕ_a and ϕ_m respectively ($\phi_m > \phi_a$ in this case), the intensity ratio of the fluorescence of the micellar solution to the aqueous solution for the same dye concentration is

† In this paper 1 M = 1 mol dm^{-3}.

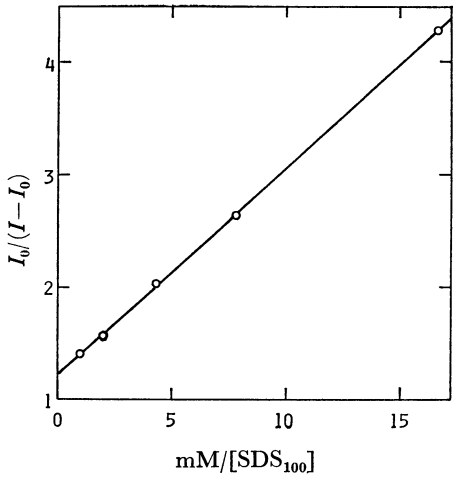

Fig. 1. The plots of $I_0/(I-I_0)$ *vs.* the inverse of the concentration of SDS micelle.
Lumiflavin, 30 μM; 0.05 M TAPS at pH 9.0 (27 °C); excitation at 440 nm; emission at 520 nm.

written as;

$$\frac{I}{I_0} = \frac{\phi_a[LF_a] + \phi_m[LF_m]}{\phi_a[LF_t]}, \quad (1)$$

where $[LF_m]$ is the partial apparent concentration of lumiflavin in the micellar phase relative to the total volume of the micellar solution, $[LF_a]$ is the concentration in the aqueous phase, and $[LF_t]=[LF_m]+[LF_a]$. On the other hand, the partition coefficient (K), which is the ratio of the local concentration of lumiflavin in the micellar phase to $[LF_a]$ (the volume of the micellar phase is negligibly small compared with the total volume), is written as;

$$K = \frac{[LF_m]/[N_a v_m[M])}{[LF_a]}, \quad (2)$$

where [M] is the molar concentration of micelle particles, v_m is the volume of a micelle, and N_a is the Avogadro constant. By combining Eqs. 1 and 2, Eq. 3 is obtained:

$$\frac{I_0}{I-I_0} = \left(\frac{\phi_a}{\phi_m-\phi_a}\right)\left(\frac{1}{KN_a v_m[M]}+1\right). \quad (3)$$

The micelle concentration [M] was estimated from the aggregation number (100)[10] and the CMC value (2.0 mM) under these conditions, assuming a constant concentration of the monomer surfactant above the CMC. Figure 1 shows that Eq. 3 holds, from which the partial concentration of lumiflavin present in the micellar phase is obtained for a given micelle concentration ($KN_a v_m = 7.0\times10^3$ M^{-1}, at 20 °C).

The highest concentration of lumiflavin examined was 270 μM under the SDS concentration of 27 mM (micelle concentration: 250 μM); then, the concentration of lumiflavin in the micelles was calculated to be 170 μM, and the concentration of lumiflavin in the bulk, to be 100 μM (nearly saturation).

Assuming the Poisson distribution of the lumiflavin molecules among the micelle particles, the fraction of micelles which contain no lumiflavin molecules is $e^{-\lambda}$, where λ is the ratio of the number of lumiflavin molecules in micelles to the number of micelle particles. Under the present conditions, where the concentration of micelles and the partial concentration of lumiflavin in the micellar phase are 250 μM and 170 μM respectively, the fraction of micelles with lumiflavin is calculated to be 0.53.

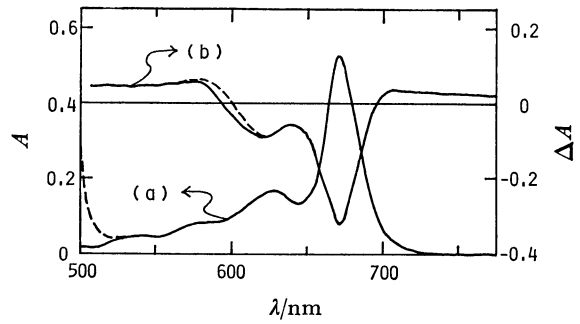

Fig. 2. The absorption spectra (a) and the transient difference spectra immediately after the flash excitation (b) of Chl a in the deaerated SDS micellar solutions with and without lumiflavin.
Chl a, 10 μM; lumiflavin, 0 mM (——) and 270 μM (----); SDS, 27 mM; 0.05 M TAPS at pH 9.0 by 200 J flash.

The local concentration of lumiflavin in the micelle particles is evaluated as 30 mM, assuming a spherical micelle with a radius of 1.8 nm, which is 300 times larger than the solubility in water and about 10 times larger than that in chloroform.

The oxygen concentration in the aerated solution with 27 mM SDS (250 μM in micelle concentration) and 50 mM TAPS at 30 °C was 233 μM, while that in the solution without SDS was 226 μM. The increase in the 7 μM oxygen content is attributable to the solubilization of oxygen in micelles. This means that only one-thirtieth of the micelles contain oxygen, on the average, although the local concentration of oxygen in micelles is calculated to be as high as 2 mM, which agrees with the solubility of oxygen in typical organic solvents.

Results and Discussion

Photolysis in Deaerated SDS Micellar Solutions. When a deaerated SDS micellar solution of Chl a without lumiflavin was irradiated by flashing light, only the triplet state of Chl a was observed as the transient species; it showed a broad absorption spectrum (Fig. 2) and decayed exponentially at the rate parameter of 1380 s^{-1}. The second-order component due to the T–T annihilation was not observed.

The addition of 270 μM lumiflavin had little effect on either the transient absorption spectrum or the initial absorbance change on the flash excitation (Fig. 2) and effected no permanent change at all. However, the kinetical behavior of the transient species was quite different; that is, the decay followed a second-order rate, as is shown in Fig. 3a. The value of $k_1/(\Delta\varepsilon d)$ is determined from the slope to be 1.0×10^4 s^{-1} at 670 nm for a 1-cm cell, where k_1 (M^{-1}S^{-1}) is the rate parameter of the second-order process, $\Delta\varepsilon$(M^{-1} cm^{-1}) is the difference in the molar absorption coefficients between the transient and final species at 670 nm, and d is the cell length in cm.

The transient absorbance change induced by the flash irradiation is considered to be due to the electron-transfer reaction from Chl a to lumiflavin in the initial stage, followed by the backward electron-transfer reaction between the Chl a cation radical and lumiflavin

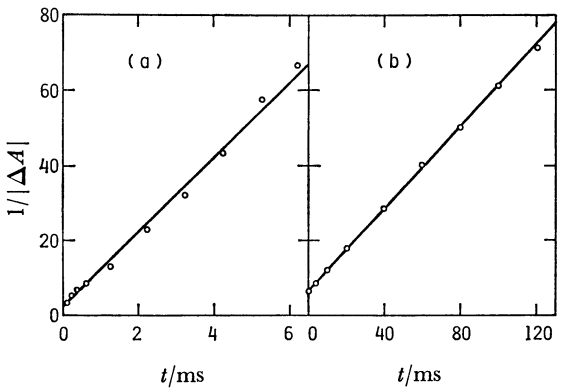

Fig. 3. Second order decay of the flash induced absorbance change of Chl a with lumiflavin in the deaerated (a) and aerated (b) SDS micellar solutions.
Chl a, 10 μM, lumiflavin, 270 μM; 0.05 M TAPS at pH 9.0 by 200 J flash, observed at 670 nm with 1-cm cell.

semiquinone, which are the products of the initial reaction. If both radicals remained in the same micelle after the initial stage, the back reaction should be first-order, contradicting the observation. Accordingly, the lumiflavin semiquinone anion radical must be immediately expelled to the bulk by the effect of the negative charge of the micelle. This second-order decay was also observed in the solution of Chl a with a lower concentration of lumiflavin (15 μM), where 97% of the Chl a molecules are in micelle particles free from lumiflavin. Therefore, the triplet state of Chl a in micelles (Chl a^T_m) can also react, within its lifetime, with the lumiflavin present in the bulk (LF_a):

$$\text{Chl } a^T_m + LF_a \longrightarrow \text{Chl } a^{+\cdot}_m + LF^{-\cdot}. \quad (4)$$

When the flash energy was increased, the initial absorbance change asymptotically reached 0.46 for 10 μM Chl a in the micellar solution with lumiflavin (270 μM). Under these limiting conditions, Chl a is completely converted to the cation radical. Therefore, $\Delta\varepsilon$ is calculated to be 4.6×10^4 M^{-1} cm^{-1}, and the rate parameter, (k_1) to be 4.6×10^8 M^{-1} s^{-1}.

The decay reaction was examined for different ionic strengths in the presence of 0-, 0.07-, and 0.13-M NaCl, and it was found that the decay was not altered. This shows that the species reacting with Chl a cation radicals in micelles is the protonated form (LFH·) of the lumiflavin semiquinone anion radical, with no charge:

$$\text{Chl } a^{+\cdot}_m + \text{LFH}\cdot \xrightarrow{k_1} \text{Chl } a_m + LF + H^+, \quad (5)$$

where k_1 is the rate parameter evaluated above. Under the present conditions (pH 9.0), it is natural that most of the lumiflavin semiquinone radicals exist in the acidic form, with no charge, in the close vicinity of micelle particles, because the pK_a value of LFH· has been reported to be 8.36[11] and the pH in the vicinity of SDS micelles is lower by 2 units than the value in the bulk.[12]

Photolysis in Aerated SDS Micellar Solutions. In an aerated SDS micellar solution, Chl a showed no absorbance change upon flash irradiation, but upon the addition of 270 μM lumiflavin, the following absorbance change was observed upon the flash excitation. The transient-difference spectrum was similar to those observed in the deaerated solutions, but the decay was much slower. The decay kinetics was also second-order as is shown in Fig. 3b, from which the slope is obtained as 540 s^{-1} at 670 nm with a 1-cm cell. The initial absorbance change for an infinite light intensity was obtained to be 0.23 at 670 nm with 10 μM Chl a and 270 μM lumiflavin, this is half of the value for the deaerated solution.

Table 1. Effect of NaCl on the decay parameter (k) of the Chl a cation radical in the aerated SDS micellar solutions

[NaCl] / M	k / 10^7 M^{-1} s^{-1}	k/k_0
0	2.5	(1)
0.055	3.5	1.41
0.104	4.7	1.88
0.179	5.4	2.16

Chl a, 10 μM; lumiflavin, 270 μM; SDS, 27 mM; 0.05 M TAPS pH 9.0.

Combining this with the finding that 53% of Chl a is present with lumiflavin in the same micelle, one may safely propose the following mechanism. The triplet Chl a in a micelle is rapidly quenched by oxygen in the aqueous phase without reacting with lumiflavin if lumiflavin is absent in the same micelle:

$$\text{Chl } a^T_m + O_2 \longrightarrow \text{Chl } a_m + O_2. \quad (6)$$

However, if present, Chl a is immediately oxidized to the cation radical and the lumiflavin semiquinone anion radical is expelled from the micelle. This anion radical will be rapidly oxidized back to lumiflavin as:

$$LF^{-\cdot} + O_2 \longrightarrow LF + O_2^{-\cdot}. \quad (7)$$

Since the pK_a value of $HO_2\cdot/O_2^{-\cdot}$ is 4.88,[13] the reduced oxygen exists exclusively as $O_2^{-\cdot}$, even in the vicinity of a micelle when the bulk pH value is 9:

$$\text{Chl } a^{+\cdot}_m + O_2^{-\cdot} \xrightarrow{k_2} \text{Chl } a_m + O_2. \quad (8)$$

Using the same $\Delta\varepsilon$ value as in the case of the deaerated solution, the rate parameter (k_2) is calculated to be 2.5×10^7 M^{-1} s^{-1}, about one-twentieth of the value for the deaerated solution. This slow back reaction of the Chl a cation radical can be explained by the repulsive action of the anionic micelle against $O_2^{-\cdot}$. This is supported by the fact that the decay parameter is increased with the concentration of sodium chloride, as is shown in Table 1.

When the dissolved oxygen concentration is reduced to below 30 μM, a fast process appears at the initial stage of the decay profile, and the initial absorbance change becomes larger (Fig. 4). The fraction of the slower component is obtained from the later stage of the decay curve; its change with the oxygen concentration is also shown in Fig. 4. In these low oxygen concentrations, the reactions in Eqs. 4 and 6 compete with each other, as do the reactions in Eqs. 5 and 7.

Photolysis in Dioxane. On the flash excitation

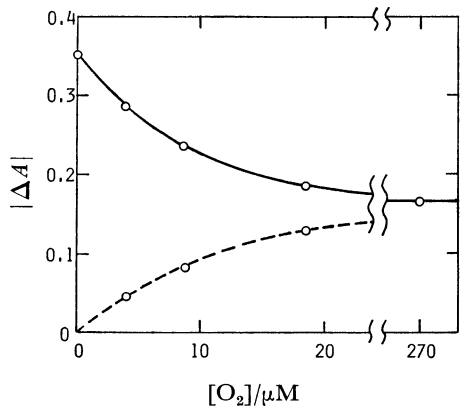

Fig. 4. Variation of the initial absorbance change of 10 μM Chl a in the presence of 270 μM lumiflavin by 200 J flash at 670 nm, with the concentration of oxygen.
Total (——); the component with the longer life time (----).

of Chl a in deaerated dioxane, the triplet state of Chl a was observed. It decayed at 700 s^{-1} at a low concentration of the triplet state, and the second-order component due to the T–T annihilation was overlapped at the early stage of the decay. (In the micellar solutions, the T–T reaction is blocked, because each Chl a is present statistically in a different micelle under the conditions examined.) On the flash photolysis of Chl a in the deaerated dioxane with 90 μM lumiflavin, the signal height and the decay were almost the same as those without lumiflavin. That is to say, neither the light-driven electron transfer reaction nor the quenching occurs in such a nonpolar solvent. In aerated solutions with and without lumiflavin, no absorbance change was observed after the flash excitation.

Photolysis in CTAB Micellar Solutions. When Chl a was irradiated by flashing light in a deaerated CTAB micellar solution, the triplet state of Chl a appeared as in the SDS solution and showed a first-order decay with 1600 s^{-1}. However, in the presence of 160 μM lumiflavin, the results were quite different from those in the SDS solutions; that is, the transient absorbance change in the deaerated solution was markedly small compared with the change in the SDS solution, and it was barely observed at all in the aerated solution. Under these conditions, lumiflavin acts mainly as quencher for the triplet state of Chl a. These results can be explained by saying that the escape of the lumiflavin semiquinone from the micelle is blocked by the positive charge of the cationic micelle.

The function of the surface charge of micelles is a dominant factor in both the formation and decay processes of the Chl a cation radical.

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