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FLUORESCENCE CHANGES RELATED IN THE PRIMARY PHOTOCHEMICAL REACTION IN THE *P*-700-ENRICHED PARTICLES ISOLATED FROM SPINACH CHLOROPLASTS

ISAMU IKEGAMI

Laboratory of Chemistry, Faculty of Medicine, Teikyo University, Ohtsuka 359, Hachioji-shi, Tokyo (Japan)

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SUMMARY

The light-induced changes in the yield of chlorophyll *a* fluorescence and photooxidation of *P*-700 in the *P*-700-enriched particles isolated from spinach chloroplasts were studied.

1. Fluorescence emitted from the particles was found to show light-induced transient changes in the yield. In the presence of ascorbate, illumination induced quenching of fluorescence in parallel to the photooxidation of *P*-700. The time course of dark reduction of photooxidized *P*-700 agreed well with that of dark recovery of variable fluorescence yield in the presence of ascorbate. When illuminated in the presence of dithionite, the emission yield increased, whereas no photooxidation of *P*-700 was observed.

2. The yield of variable fluorescence and redox state of *P*-700 depended similarly upon the redox potential.

3. At liquid nitrogen temperature, illumination induced a rise of the fluorescence yield and a complete photooxidation of *P*-700 in the ascorbate-treated sample. When the particles had been preincubated with dithionite in the light before cooling, light-induced rise in the fluorescence yield was accompanied by only a small extent of *P*-700 photooxidation. It is suggested that both the oxidized form of *P*-700 and the primary electron acceptor act as quenchers for the variable fluorescence.

4. The emission spectrum for the constant part of fluorescence (F679) has a peak at 679 nm, and that for the variable part of fluorescence (F694) has a peak at 694 nm at room temperature. The emission maxima were slightly shifted and the yield of variable fluorescence was markedly enhanced at liquid nitrogen temperature.

5. Excitation spectra determined show a peak at 672 nm for F679, and a peak at 672 nm and a shoulder at 685 nm for F694. Action spectrum for *P*-700 photooxidation was similar to the excitation spectrum for F694.

INTRODUCTION

In higher plants and algae, fluorescence of chlorophyll *a* is emitted mostly from chlorophyll *a* belonging to Photosystem II at room temperature [1–4]. Evidence has

been accumulated to indicate that chlorophyll fluorescence serves as an intrinsic probe for the photochemical events related with Photosystem II [3, 4]. On the other hand, knowledge about fluorescence emitted from pigments of Photosystem I is still scarce. An induction phenomenon of fluorescence which would reflect the primary events of photochemical reaction center 1 has not yet been reported [5–7].

In the previous report, the preparation of Photosystem I particles which contained one *P*-700 for every 6 to 9 chlorophyll molecules has been described [8]. Because of its low pigment content, the *P*-700-enriched particle is especially suitable for the spectrophotometric investigation of the primary photochemistry of Photosystem I.

In the present work, the light-induced changes in fluorescence yield and the photooxidation of *P*-700 were comparatively studied with the particles. It will be shown that on illumination, fluorescence of Photosystem I shows transient changes in the emission yield. Evidence will be presented to indicate that the yield of the variable fluorescence is regulated by the redox states of the primary electron donor and acceptor of Photosystem I. In addition, roles of chlorophyll *a* forms in the particles in the fluorescence emission and energy transfer to the reaction center were analyzed.

MATERIALS AND METHODS

The *P*-700-enriched particles were prepared as described previously [8]. In brief, spinach chloroplasts suspended in 0.4 M sucrose, 50 mM phosphate buffer (pH 7.8), 10 mM KCl and 5 mM $MgCl_2$, were incubated with 5 % digitonin for 1 h at 0–4 °C. The ratio of digitonin to chlorophyll was about 10. The suspension was then subjected to centrifugal fractionation. The particles obtained between $125\,000 \times g$ (20 min) and $313\,900 \times g$ (50 min) were found to have chlorophyll *a*/*b* ratios of 7.0–8.5, which are significantly higher than that of D144 particles of Anderson and Boardman [9], or Wessels [10]. The particles with high *a*/*b* ratios were washed twice with deionized water and, after lyophilization, extracted twice with ether containing 75 % saturation water (0–4 °C). The ratio of *P*-700 to chlorophyll *a* in the *P*-700-enriched particles employed in the present work was about 8.

Chlorophyll was determined by the method of Arnon [11].

The measurements of fluorescence were performed as described previously [12]. The blue excitation light, supplied with 650 W halogen lamp, passed through an infrared-absorbing filter (Hoya HA-50), two blue filters (Hoya B-440 and Corning 4-96) and a water layer of 5 cm thickness. The light intensity determined with Kipp and Zonen thermopile was $2 \cdot 10^4$ erg $\cdot$ cm$^{-2} \cdot$ s^{-1}. Fluorescence emitted from the sample was analyzed using a Nikon G-250 grating monochrometer (usually half band width; 3.2 nm) and detected by a photomultiplier (Hamamatsu TV R636). The emission spectra corrected for the spectral sensitivity were determined with the aid of the optical set-up constructed by Dr. N. Murata [13].

The absorption spectra and the photooxidations of *P*-700 were determined with a Hitachi 356 Dual Wavelength spectrophotometer, as described previously [8]. The actinic light system was the same as that described for the fluorescence excitation.

RESULTS

Fig. 1 shows the time courses of changes in the yield of chlorophyll *a* fluorescence in the *P*-700-enriched particles at room temperature (approx. 15 °C). There was no time-dependent change in the yield, when fluorescence was observed at 665 nm. Neither ascorbate nor dithionite had any appreciable effect on the yield or the time courses of changes of the emission at this wavelength. On the other hand, the yield of fluorescence determined at 694 nm was found to vary during illumination. Without any addition, illumination induced a slight decrease in the fluorescence yield at 694 nm. The variable part of fluorescence was eliminated by the addition of ferricyanide (data not shown). When the particles had been incubated with ascorbate, the initial level of fluorescence intensity increased and the subsequent quenching became more marked. The initial fluorescence level was further elevated in the particles which had been preincubated with dithionite. In this case, however, the fluorescence yield rose to a higher final level during the illumination.

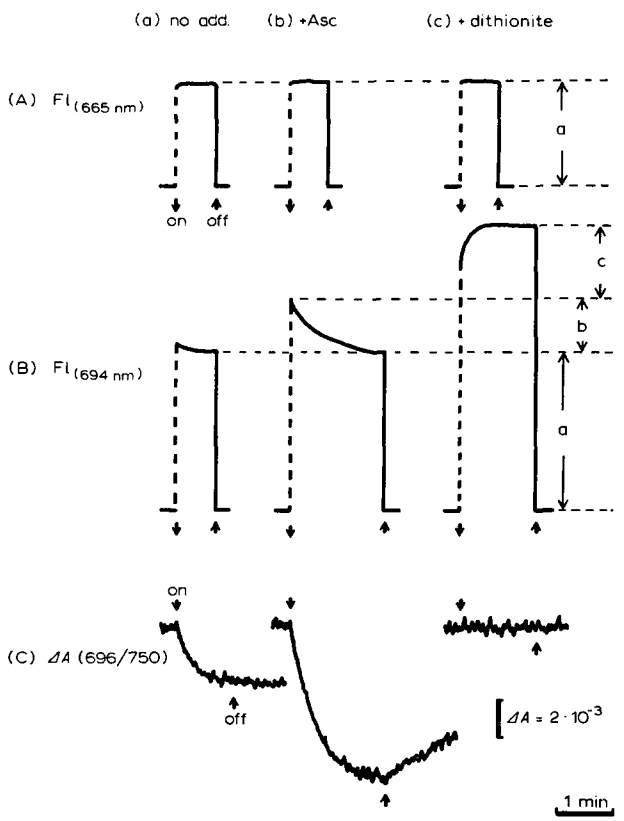

Fig. 1. Time courses of changes in the fluorescence yield and photooxidation of *P*-700 at room temperature. Time courses of fluorescence changes were determined at 665 mn (A) or at 694 nm (B). *P*-700 photooxidation (C) was determined at 696 nm with a reference wavelength at 750 nm. Where indicated, the particles were incubated with 5 mM ascorbate or 5 mM dithionite for about 10 min prior to illumination. For experimental details, see Methods.

Fig. 1 also shows time courses of the light-induced changes of *P*-700. Without any addition, illumination induced only a small extent of *P*-700 photooxidation. The photooxidation was completely suppressed by the addition of ferricyanide. In the particles pretreated with ascorbate, the extent of *P*-700 photooxidation increased markedly, indicating that most of *P*-700 was in the oxidized state in the particles. The slow rate of *P*-700 photooxidation (and that of fluorescence decrease) would be a reflection of a situation where most of the light-harvesting chlorophyll was absent from the particles and the light intensity employed was far below saturation. The addition of dithionite completely eliminated the photooxidation of *P*-700. Presumably, the rate of *P*-700 reduction with dithionite exceeds the rate of *P*-700 photooxidation.

The above observation that the extent and time courses of the light-induced quenching of fluorescence were similar to those of *P*-700 photooxidation in the absence and presence of ascorbate suggests that the yield of variable fluorescence at 694 nm is determined by the redox state of *P*-700. Results strongly supporting this view were obtained in the following experiments in which correlation between the fluorescence yield and redox state of *P*-700 was studied.

The particles were illuminated to oxidize *P*-700 completely and then left in the dark in the presence of 5 mM ascorbate for varied periods of time. In Fig. 2, the extent of *P*-700 photooxidation on the second illumination were plotted against the dark time after the first illumination. It was found that *P*-700 was only slowly reduced with ascorbate and that the complete reduction of *P*-700 was attained after 7 min of dark incubation. Similar experiments were carried out to determine the time course of the dark recovery of the variable fluorescence yield in the presence of ascorbate. The two time-course curves agree well each other. On the other hand, in the absence of

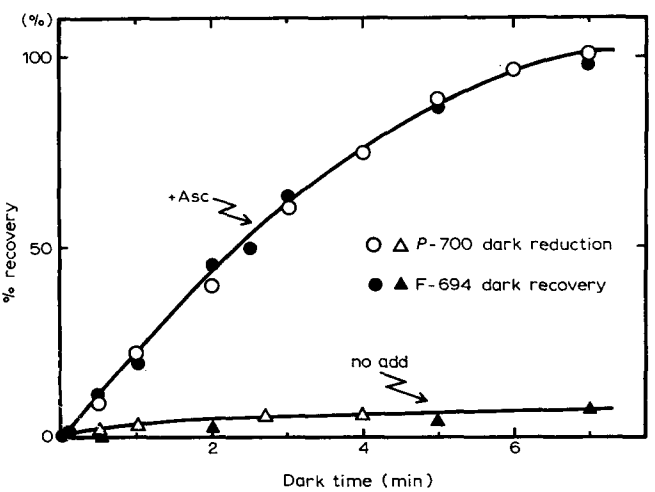

Fig. 2. Time courses of the dark recovery of variable part of fluorescence (solid symbols) and of reduction of *P*-700 (open symbols). The samples were firstly illuminated in order to quench the variable fluorescence or to oxidize *P*-700 completely and then incubated in the dark in the absence (triangles) or in the presence of 5 mM ascorbate (circles). After dark times indicated in abscissa, the second illumination was given and intensities of the variable fluorescence or extents of *P*-700 photooxidation were determined. The magnitudes of the second illumination-induced changes were presented as percentages of those of the first illumination-induced changes.

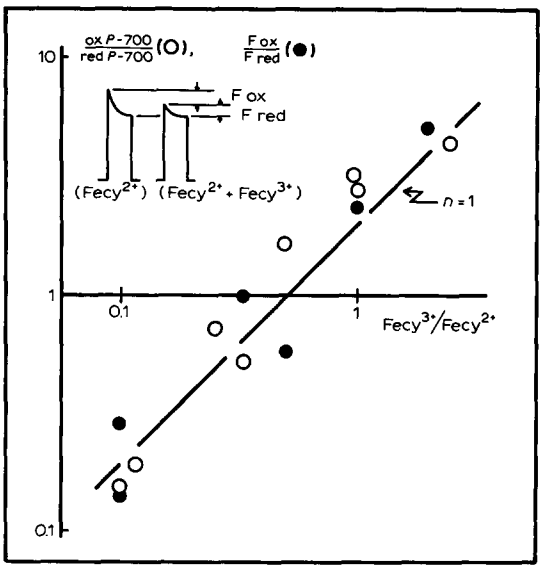

Fig. 3. Redox titrations of variable fluorescence yield (●) and *P*-700 (○). Redox titrations were carried out as described previously [14]. After the incubation of the particles with various ratios of ferri-ferrocyanide mixture, the yield of variable fluorescence was determined. The values for 0 and 100 % yield were determined with samples incubated with ferricyanide (1–5 mM) and ascorbate (5 mM), respectively. A value of 419 mV was used for the normal redox potential of ferri-ferrocyanide couple [15]. The straight line in the figure is the theoretical curve with one electron transfer reaction.

ascorbate, both the reduction of *P*-700 and the recovery of variable fluorescence were insignificant. This, together with the kinetics of light-induced changes shown in Fig. 1, strongly indicates that the reduced form of *P*-700 increases the yield of the variable fluorescence at 694 nm, whereas its oxidized form quenches the emission yield.

If the above assumption is correct, the fluorescence yield should vary, depending upon the redox potential, as does the redox state of *P*-700. This was found to be the case. Fig. 3 shows that the yield of the variable fluorescence and the reduced part of *P*-700 go in parallel with varied ratios of ferri-ferrocyanide couple in the medium, giving a midpoint potential of around 400 mV. It is concluded, therefore, that the yield of the variable fluorescence at 694 nm is proportional to the fraction of *P*-700 in the reduced state.

The light-induced increase in fluorescence yield observed in the presence of dithionite cannot be accounted for by the redox change of *P*-700, since the reductant kept *P*-700 in the reduced state during the illumination. We propose, therefore, that the primary electron acceptor for Photosystem I (designated as Z) acts also as a fluorescence quencher in its oxidized state. Similar quenching effect of the oxidized primary acceptor (Q) has been assumed for fluorescence from Photosystem II [1–4]. The light-induced increase in the fluorescence yield then reflects the reduction of Z. Accumulation of reduced Z during illumination does not occur unless dithionite prevents the reduced Z from reoxidation by oxygen through its action from consuming all the oxygen in the reaction medium. It is also assumed that dithionite cannot

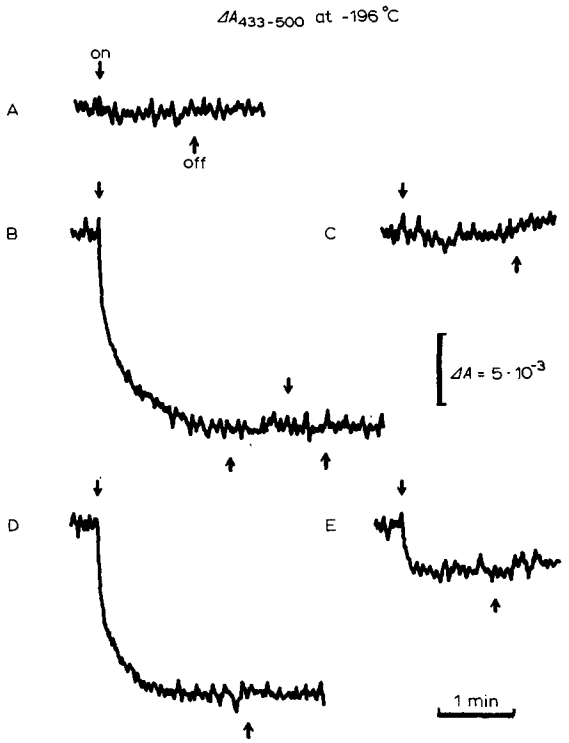

Fig. 4. Light-induced absorption changes of *P*-700 at liquid nitrogen temperature. Photooxidation of *P*-700 was determined at 433 nm with reference wavelength at 500 nm. The red excitation light was supplied with 650 W halogen lamp through two red cut-off filters (Toshiba VR-65 and VR-67), a neutral density filter (Hoya N-10) and a water layer of 5 cm thickness. The incident light-intensity was the same to that of blue excitation light described in Methods. A blue filter (Corning 4-96) was inserted in front of photomultiplier to prevent scattered excitation light. Samples were preincubated in the dark with 1 mM ferricyanide (Curve A), 10 mM ascorbate (Curve B), or a few grains of dithionite (Curve D) before cooling. Curves C and E were determined with the samples which were illuminated with excitation light during the incubation with 10 mM ascorbate or a few grains of dithionite, respectively.

reduce Z chemically. Evidence supporting this assumption was obtained from kinetical studies performed at liquid nitrogen temperature.

Fig. 4 shows time courses of *P*-700 photooxidation at liquid nitrogen temperature. In order to avoid the interference of fluorescence which was markedly intensified at low temperature, *P*-700 was determined at 433 nm. It was confirmed by the spectral studies of the variously treated samples, a part of which is presented in Fig. 4, that only *P*-700 responded to illumination at this low temperature. When the particles were incubated with ferricyanide before cooling, no absorption change was observed on illumination (Fig. 4A). When *P*-700 was reduced by the dark incubation with ascorbate before cooling, however, a large extent of *P*-700 photooxidation occurred on illumination (Fig. 4B). There was no reduction of *P*-700 in the following dark period and the second illumination gave no additional change. When the incubation with ascorbate was made under light condition, *P*-700 photooxidation disappeared at the

low temperature (Fig. 4C), since, as indicated in Fig. 1, *P*-700 was already in the oxidized state before cooling. Fig. 4D shows the occurrence of the *P*-700 photooxidation in the particles pretreated with dithionite in the dark. This supports the previous assumption that the primary electron acceptor, Z, cannot be reduced chemically by dithionite at room temperature. The fact that there was no dark reduction of photooxidized *P*-700 indicates that dithionite cannot reduce the photooxidized *P*-700 at low temperature. In contrast to the case with ascorbate, the photooxidation of *P*-700 was observed at low temperature with the samples which had been incubated with dithionite in the light prior to the cooling. However, it should be noted that as compared with the dark incubated samples, the extent of the photooxidation was markedly reduced after the light incubation.

In order to study the redox state of *P*-700 at this state, absorption spectra in the red band region of chlorophyll were determined at low temperature with variously treated particles (Fig. 5). Curve A shows the spectrum of the particles which had been incubated with dithionite in the dark or light before cooling. A shoulder around 700

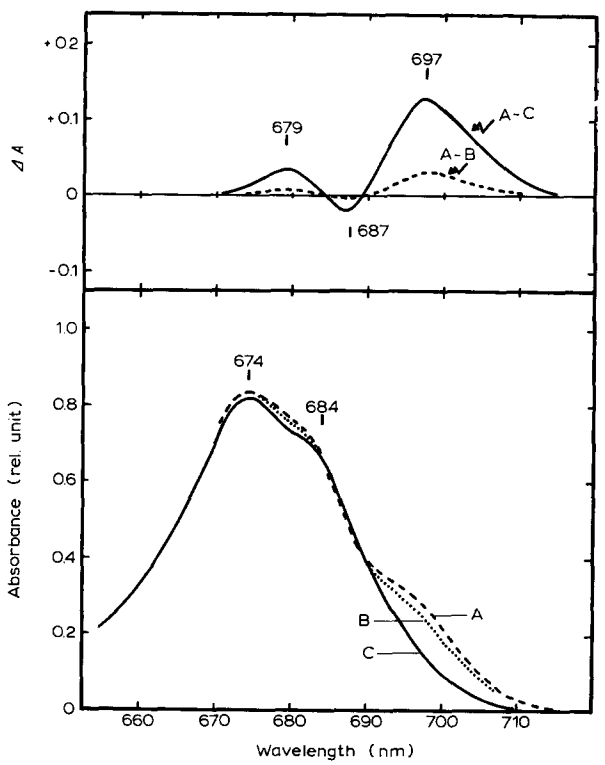

Fig. 5. The absorption spectra in red band region of *P*-700-enriched particles at liquid nitrogen temperature. Curve A: the sample was incubated in the dark with ascorbate, or dithionite before cooling. Curve B: the sample was illuminated with excitation light during the incubation with dithionite at room temperature and, after freezing, illuminated with excitation light. Curve C: the sample was incubated in the dark with 1 mM ferricyanide before cooling. Spectra similar to Curve C were also obtained with the samples which were incubated in the dark with ascorbate or dithionite at room temperature and then illuminated after cooling.

nm indicates that *P*-700 remained in the reduced state after the treatments. With the dark incubated samples, the shoulder at 700 nm completely disappeared on illumination at low temperature. The resulting spectrum (Curve C) is identical to that of ferricyanide-treated samples, indicating that *P*-700 is completely photooxidized. The difference spectrum between Curve A and Curve C is depicted in the upper part of Fig. 5. The spectrum showing a main peak at 697 nm and a satellite peak at 679 nm is not so different from the spectrum of *P*-700 at room temperature (see Fig. 1 in ref. 8), though a trough between the two peaks is very pronounced. On the other hand, a spectrum (Curve B) which was obtained by illuminating the light-incubated samples, shows that a large portion of *P*-700 is still in the reduced state. This strongly supports the assumption that reduced Z is accumulated during illumination at room temperature in the presence of dithionite.

The light-induced changes in the yield of fluorescence at 697 nm were determined at low temperature and the results are depicted in Fig. 6. The ferricyanide-treated particles showed no variable fluorescence. After the incubation in the dark with ascorbate, the initial level of fluorescence rose to some extent, reflecting the reduction of *P*-700. Of interest is a finding that illumination induced a further rise of the emission yield. This is in contrast to the light-induced quenching observed at room temperature, in spite of the fact that *P*-700 was photooxidized at low temperature, as well as at room temperature. It is assumed, therefore, that the oxidized form of Z is a stronger quencher than the oxidized *P*-700, i.e., the fluorescence yield is higher with a pair of oxidized *P*-700 and reduced Z ($P^+ \cdot Z^-$) than with a pair of reduced *P*-700 and oxidized Z ($P \cdot Z$). The highest level of fluorescence yield thus corresponds to the state of the reaction center containing reduced *P*-700 and reduced Z ($P \cdot Z^-$). This is realized by illuminating the sample which has been preincubated with dithionite in the light at room temperature (Curve C).

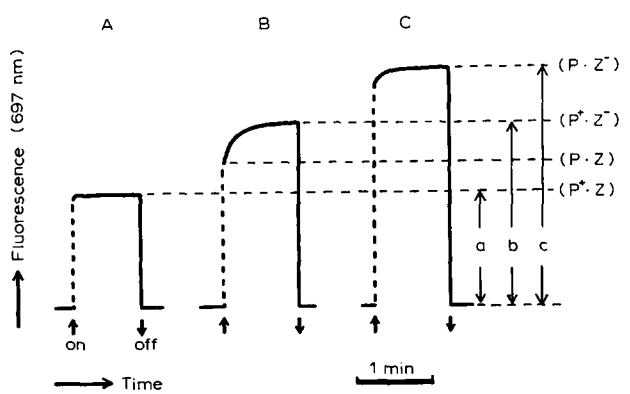

Fig. 6. Time courses of changes in fluorescence yield at liquid nitrogen temperature. Fluorescence was determined at 697 nm. The sample was incubated in the dark with 1 mM ferricyanide (Curve A), or 10 mM ascorbate (Curve B) before cooling. Curve C was obtained with the sample preilluminated with the excitation light during the incubation with dithionite (cf. Fig. 4E) before cooling. When the sample was incubated with dithionite in the dark, the time course similar to Curve B was obtained. The steady state level in Curves A, B and C correspond to the redox state of the reaction center of $P^+ \cdot Z$, $P^+ \cdot Z^-$ and $P \cdot Z^-$, respectively. The initial level in Curve B was of $P \cdot Z$. For details, see the text.

Figs. 7 and 8 show the emission spectra of fluorescence determined at room and liquid nitrogen temperature, respectively. Curve A in Fig. 7 is the emission spectrum for the constant part of fluorescence determined in the presence of ferricyanide. The emission maximum lies at 679 nm with a broad shoulder at 735 nm. This component of fluorescence will be designated as F679. The wavelength-dependency of the initial level for the fluorescence determined after the dark incubation with ascorbate is shown in Curve B in Fig. 7. The emission spectrum of the variable part of fluorescence, which was indicated as 'b' in Fig. 1, is obtained by subtracting Curve A from Curve B. The spectrum thus obtained (Curve B—A in the upper part of Fig. 7) has a peak at 694 nm with a shoulder at 740 nm. This will be designated as F694. It is obvious that F694 is not originated from reduced *P*-700, since the absorption maximum of *P*-700 in the particles is at 696 nm (see ref. 8). Curve C in Fig. 7 shows the final steady-state level of fluorescence yield attained in the presence of dithionite. The difference spectrum between Curve C and Curve A (Curve C—A) has the spectral characteristics similar to that of Curve B—A, indicating that dithionite enhanced the yield of F694.

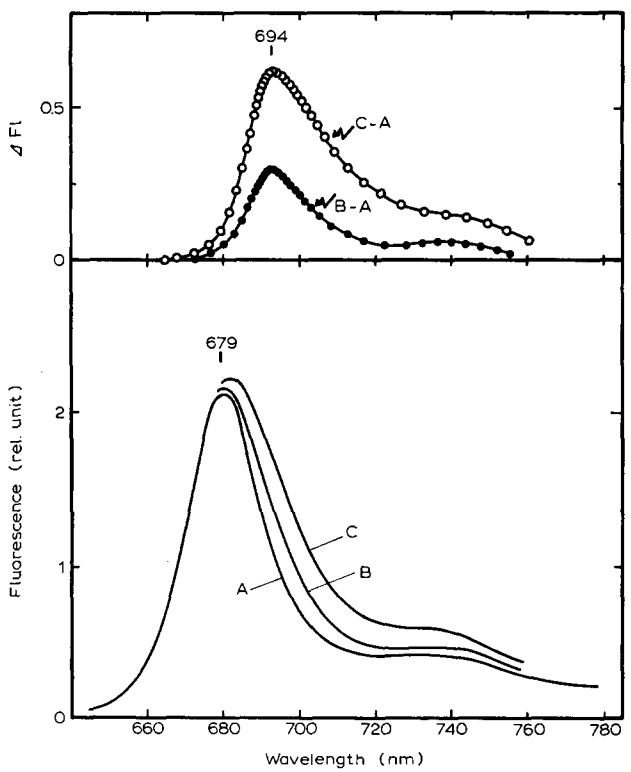

Fig. 7. Emission spectra of fluorescence determined at room temperature. Curve A: fluorescence yield in the presence of 1 mM ferricyanide. Curve B: the initial level of fluorescence determined after the dark incubation with 5 mM ascorbate. The steady state level of fluorescence attained after prolonged illumination in the presence of ascorbate shows the same spectrum as Curve A. Curve C: the steady-state level of fluorescence attained in the presence of dithionite. Curves B—A and C—A are difference spectra between Curve B and Curve A, and Curve C and Curve A, respectively.

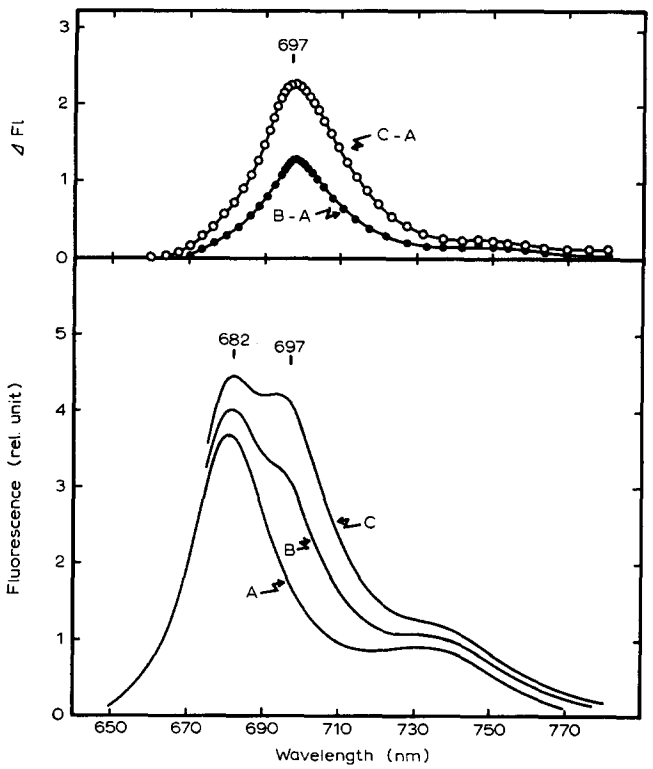

Fig. 8. Emission spectra of fluorescence determined at liquid nitrogen temperature. Curves A, B and C are the spectral dependency of 'a', 'b' and 'c' in Fig. 6, respectively. Curves B—A and C—A are difference spectra between Curve B and Curve A, and Curve C and Curve A, respectively.

The emission maxima were slightly shifted and yield of variable fluorescence was markedly enhanced at liquid nitrogen temperature (Fig. 8). The emission spectrum for the constant fluorescence (F679 at room temperature) has a peak at 682 nm with a shoulder at 735 nm, at low temperature. Emission spectra determined after the dark incubation with ascorbate, or dithionite (Curve B), or after the light incubation with dithionite (Curve C) show, besides the main emission peak at 682 nm, a distinct shoulder at around 697 nm. The difference spectra obtained by subtracting Curve A from Curve B and from Curve C (Curves B—A and C—A) show the same spectral features with a peak at 697 nm and a shoulder around 750 nm. This may correspond to F694 at room temperature.

Finally, we have studied what forms of chlorophyll *a* are responsible for the emission of the two species of fluorescence (F679 and F694) by determining the excitation spectra of fluorescence. As shown in Fig. 9, the excitation spectrum for F679 has a single peak at 672 nm. Excitation spectrum for the variable fluorescence (F694) was determined as the differences between the spectra for the final steady-state fluorescence yield attained in the presence of dithionite and that for the emission yield in the presence of ferricyanide. The resulting spectrum shows a peak at 672 nm and a shoulder at 685 nm. Fig. 9 also shows the action spectrum for the *P*-700 photooxida-

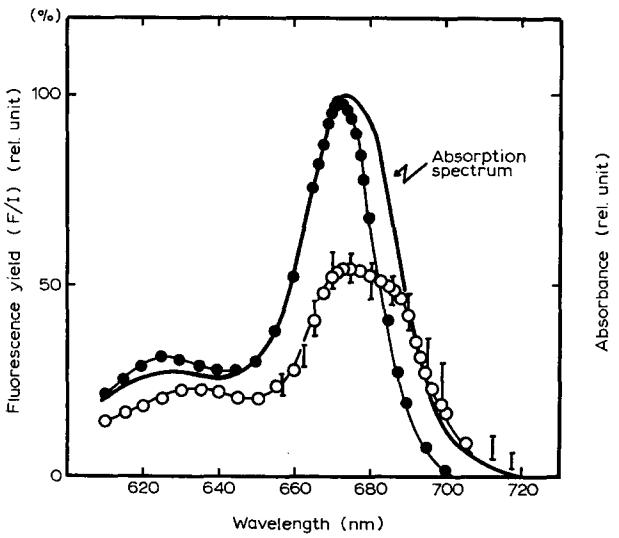

Fig. 9. Excitation spectra for the constant (solid circles) and variable (open circles) part of fluorescence and action spectrum for *P*-700 photooxidation (vertical bars). Fluorescence emitted at the longer wavelength than 720 nm was measured. Excitation monochromatic light was obtained from a 650 W halogen lamp which passed through a Nikon G-250 Grating Monochrometer (half band width, approx. 12 nm), a red cut-off filter (Toshiba VR-60), an infrared-absorbing filter (Hoya HA-50) and a water layer of 5 cm thickness. A far-red cut-off filter (Corning 7-96), which passes fluorescence at wavelengths longer than 720 nm, was placed in front of the photomultiplier. The fluorescence intensities were corrected for the incident light-intensities. Solid circles: fluorescence yield determined in the presence of 1 mM ferricyanide. Open circles: difference between the fluorescence yield determined at the stationary level attained with long illumination in the presence of dithionite and that determined in the presence of ferricyanide. The vertical bars shows the rates of *P*-700 photooxidation determined in the presence of 10 mM ascorbate and at 433 nm (reference wavelength, 460 nm) with the same monochromatic excitation light as used for the fluorescence measurements. They were normalized for the excitation light-intensities. For the comparison, the vertical bars and open circles were normalized at 675 nm. The solid line: the absorption spectrum in the oxidized state of the *P*-700-enriched particles used in the experiment. The absorption spectrum was normalized with solid circles at 672 nm.

tion. It agrees well with the excitation spectrum for F694. However, it is to be noted that, at longer wavelength region, the action spectrum is similar to the absorption spectrum of the reduced samples, whereas the excitation spectrum for F694 is close to that of oxidized samples. This indicates that light absorbed by reduced *P*-700 is also effective in sensitizing photooxidation of *P*-700.

DISCUSSION

The present work first demonstrated that fluorescence emitted from Photosystem I chlorophyll *a* shows an induction phenomenon on illumination, i.e. the fluorescence yield shows transient changes until it reaches to the final steady state. The previous failure in detecting the fluorescence induction in Photosystem I must be due to the very low intensity of the variable fluorescence. Since chloroplasts or the Photosystem I particles contain larger amounts of bulk chlorophyll than the particles

employed here, the variable fluorescence would be virtually buried in the noise level. Even in the *P*-700-enriched particles, the intensity of variable fluorescence is several fold weaker than that of the constant fluorescence.

Evidence has been accumulated indicating that the yield of chlorophyll fluorescence in photosynthetic organisms varies in response to changes in the reaction center, electron transport system, energy state or the structure of photosynthetic membranes or ion transport across the membrane. However, since the reaction center of Photosystem I is the sole component which responds to illumination in the *P*-700-enriched particles, the light-induced change in the fluorescence yield should be ascribed to changes in the redox state of the reaction center.

The results obtained in the present work cannot be explained by the simple model that the fluorescence yield is inversely proportional to the photochemistry in the reaction center. This model predicts that the fluorescence yield is high when the reaction center is closed, and low when it is open. This is proved not to be the case. The yield of the variable fluorescence is negligible when the reaction center is closed with the primary electron donor and acceptor both in the oxidized state. On the other hand, the highest yield is obtained with the closed reaction center, the donor and acceptor of which are both in the reduced state. Furthermore, the kinetic studies show that under the condition where reoxidation of the reduced acceptor is rapid, the emission yield is determined solely by the portion of *P*-700 in the reduced state (Figs. 2, 3). On the other hand, when reoxidation of the acceptor is prevented at liquid nitrogen temperature, or by the addition of dithionite at room temperature, the yield is also affected by the redox state of the acceptor. Thus, the present observation can be best explained by assuming that oxidized donor and acceptor act as quenchers for the variable fluorescence. The oxidized acceptor is more effective in quenching fluorescence than oxidized *P*-700, since reduction of the acceptor coupled with oxidation of *P*-700 is accompanied by the increase in the emission yield (Fig. 6).

No evidence was obtained to indicate that the variable fluorescence is emitted from reduced *P*-700. The emission maximum of the variable part of fluorescence (694 nm) lies at shorter wavelength than the absorption maximum of *P*-700. In addition, it was found that the yield of fluorescence increased when the absorption peak around 700 nm disappeared (Figs. 4 and 6). It is concluded, therefore, that the variable part of fluorescence is not emitted from reduced *P*-700, but is originated from a form of chlorophyll *a* which is closely associated with the reaction center of Photosystem I.

The mechanism proposed above that both the primary electron donor and acceptor quench fluorescence in the oxidized state is very similar to the mechanism which has been proposed for the regulation of fluorescence yield in Photosystem II [1–4, 16–21]. Duysens and Sweers [1] showed that fluorescence yield is high when the primary electron acceptor, Q, of Photosystem II is reduced, whereas the oxidized Q quenches fluorescence. The fluorescence yield in Photosystem II is also affected by the redox states of primary electron donor of Photosystem II [16–21].

It is also well established that in the highly purified reaction center preparations from photosynthetic bacteria, the fluorescence yield is determined by the redox states of the primary electron donor and acceptor [22]. In contrast to the case with Photosystem I, however, the variable part of fluorescence is emitted from the reaction center bacteriochlorophyll.

The constant and variable fluorescence emitted from the *P*-700-enriched

particles were found to have different emission spectra, indicating that they are originated from different forms of chlorophyll *a*. The absorption spectra of the particles determined at room and low temperature indicate that the particles contain three forms of chlorophyll, chlorophyll-*a*-672, chlorophyll-*a*-685 and *P*-700, in the order of abundance. The preparations employed in this work have about 8 chlorophylls for every *P*-700. Assuming that *P*-700 is a chlorophyll dimer and that the absorption spectrum of chlorophyll-*a*-672 is identical to the action spectrum for F679 (Fig. 9), it is estimated that the quantitative ratio of chlorophyll-*a*-672, chlorophyll-*a*-685 and *P*-700 in the particles is 5 : 2 : 1. The chlorophyll to *P*-700 ratios varied somewhat with the particles preparations. The ratio of chlorophyll-*a*-685 to *P*-700 was found to be fairly constant among various preparations, whereas chlorophyll-*a*-672 to *P*-700 ratio was variable between 2 and 6. This suggests that chlorophyll-*a*-685 is more closely associated with the reaction center than chlorophyll-*a*-672. Most likely, F694 is emitted from chlorophyll-*a*-685. The excitation spectrum for F694 indeed indicates the presence of shoulder at 685 nm (Fig. 9). The excitation spectrum for F679 shows a single peak having a maximum at 672 nm, indicating that F679 is emitted from chlorophyll-*a*-672 (Fig. 9). The excitation spectrum for F694 and the action spectrum for the *P*-700 photooxidation show the participation of chlorophyll-*a*-672 in both reaction. However, the efficiency of energy transfer from chlorophyll-*a*-672 to other forms of chlorophyll is low. It can be estimated in comparing the absorption spectrum with the action spectra (Fig. 9) that about a half of the chlorophyll-*a*-672 molecules transfer their excitation energy to chlorophyll-*a*-685 or *P*-700.

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REFERENCES

1 Duysens, L. N. M. and Sweers, J. E. (1963) in Studies on Microalgae and Photosynthetic Bacteria (Japan Soc. Plant Physiol.), pp. 353–372, University of Tokyo Press, Tokyo
2 Butler, W. L. and Bishop, N. I. (1963) in Photosynthetic Mechanisms of Green Plants (Kok, B. and Jagendorf, A. T., eds.), pp. 91–100, National Academy of Sciences, Washington
3 Murata, N., Nishimura, M. and Takamiya, A. (1966) Biochim. Biophys. Acta 120, 23–33
4 Malkin, S. and Kok, B. (1966) Biochim. Biophys. Acta 126, 413–432
5 Boardman, N. K., Thorne, S. W. and Anderson, J. M. (1966) Proc. Natl. Acad. Sci. U.S. 56, 586–593
6 Ke, B. and Vernon, L. P. (1966) Biochemistry 6, 2221–2226
7 Boardman, N. K. and Thorne, S. W. (1969) Biochim. Biophys. Acta 189, 294–297
8 Ikegami, I. and Katoh, S. (1975) Biochim. Biophys. Acta 376, 588–592
9 Anderson, J. M. and Boardman, N. K. (1966) Biochim. Biophys. Acta 112, 403–421
10 Wessels, J. S. C. (1966) Biochim. Biophys. Acta 126, 581–583
11 Arnon, D. I. (1949) Plant Physiol. 24, 1–15

12 Ikegami, I. and Katoh, S. (1972) Arch. Biochem. Biophys. 150, 9–14
13 Murata, N., Nishimura, M. and Takamiya, A. (1966) Biochim. Biophys. Acta 126, 234–243
14 Ikegami, I., Katoh, S. and Takamiya, A. (1968) Biochim. Biophys. Acta 162, 604–606
15 O'Reilly, J. E. (1973) Biochim. Biophys. Acta 292, 509–515
16 Okayama, S. and Butler, W. L. (1971) Biochim. Biophys. Acta 234, 381–389
17 Joliot, P. and Joliot, A. (1972) in IInd International Congress on Photosynthesis Research (Forti, G., Avron, M. and Melandri, A., eds.), Vol. 1, pp. 26–38, Dr. Junk N.V., The Hague
18 Mauzerall, D. (1972) Proc. Natl. Acad. Sci. U.S. 69, 1358–1362
19 Butler, W. L., Visser, Jan W. M. and Simons, H. L. (1973) Biochim. Biophys. Acta 292, 140–151
20 Butler, W. L., Visser, Jan W. M. and Simons, H. L. (1973) Biochim. Biophys. Acta 325, 539–545
21 Den Haan, G. A., Duysens, L. N. M. and Egberts, D. J. N. (1974) Biochim. Biophys. Acta 368, 409–421
22 Zankel, K. L., Reed, D. W. and Clayton, R. K. (1968) Proc. Natl. Acad. Sci. U.S. 61, 1243–1249