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FURTHER CHARACTERIZATION OF A PHOTOSYSTEM II PARTICLE ISOLATED FROM SPINACH CHLOROPLASTS BY TRITON TREATMENT

DELAYED LIGHT EMISSION *

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Summary

Delayed light emission from the Triton-fractionated Photosystem II subchloroplast fragments (TSF-IIa) was measured between 0.5 and 10 ms after the termination of illumination. The delayed light emission was diminished by Photosystem II inhibitors, DCMU and *o*-phenanthroline, which act between the reduced primary acceptor and the plastoquinone pool.

Secondary electron donors to Photosystem II, diphenylcarbazide, phenylenediamine, Mn^{2+}, and ascorbate inhibited delayed light emission. Secondary electron acceptors such as ferricyanide, dichlorophenol indophenol, and dimethyl benzoquinone enhanced delayed light emission. The addition of secondary electron acceptors to TSF-IIa particles containing Mn^{2+} restored delayed light emission to almost the control level. The plastoquinone antagonist, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, increased delayed light emission at low concentrations but decreased it at higher concentrations. Silicomolybdate enhanced the delayed light emission of TSF-IIa particles markedly, and reversed the inhibition by DCMU. Silicomolybdate showed a similar stimulatory effect on the delayed-light intensity in broken spinach chloroplasts at shorter times after the termination of illumination. Carbonyl cyanide *m*-chloro (or *p*-trifluoromethoxy) phenylhydrazones inhibited the delayed light emission, but NH_4Cl had no effect.

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Abbreviations: TSF-IIa, Triton-fractionated Photosystem II subchloroplast fragment with a high chlorophyll *a* content; Chl, chlorophyll; DCIP, 2,6-dichlorophenol indophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DPC, diphenylcarbazide; MES, 2-(*N*-morpholino)ethanesulfonic acid; TES, *N*-tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

All above observations are consistent with the TSF-IIa particles consisting of Photosystem II reaction centers, and with the currently accepted model of recombination of charged primary donor and acceptor as the origin of delayed light emission.

Introduction

A small, 10 nm-diameter particle, designated as TSF-IIa, with a high Chl *a*/*b* ratio and Photosystem II activity was first reported by Vernon et al. [1]. These particles have the following characteristics indicative of Photosystem II: highly enriched in cytochrome *b*-559, which can be photoreduced at room temperature with or without an exogenous electron donor [2]; a simple fluorescence emission spectrum with a single peak at 680 nm at room temperature and 685 nm at 77 K [2]; and fluorescence induction phenomena [3].

Subsequent reexamination of the Chl *a*/*b* ratio yielded a value of 28 ± 2 [3]. The then current candidates for the primary electron donor and acceptor of Photosystem II, *P*-680 and C-550, respectively, were also detected by the measurement of low-temperature light-induced difference spectra [3]. The presence of *P*-680 was further corroborated by EPR spectroscopy [3].

The Photosystem II particles contain no detectable *P*-700 or bound iron-sulfur proteins [3]. It was also shown that the particle can recombine with the Photosystem I particles with good reconstitution activity [4].

Vernon et al. [5] also reported that these particles emit delayed light. Since delayed light emission is characteristic of Photosystem II, it is of interest to further characterize the delayed light emission in these particles.

Materials and Methods

The TSF-IIa particles were prepared from spinach chloroplasts as described earlier [2,5]. These particles can photoreduce DCIP at a high rate (≈500—1000 μmol/mg Chl per h) with DPC as donor. The particles were stored deep-frozen at −80°C until needed.

Millisecond-delayed light was measured with a Becquerel phosphoroscope [6]. The instrument consists of a pair of rotating discs mounted on a common shaft, with the appropriately spaced holes on the discs. The instrument was modified to decrease the illumination time and increase the time during which delayed light emission was measured. At the usual rate of rotation of the discs, the illumination time was 0.5 ms and delayed emission was measured from 0.5 to 10 ms after illumination.

The decay kinetics of delayed light emission were measured by an EMI 9558 photomultiplier. The photomultiplier output was first recorded by a Biomation Model 610 transient recorder and then transcribed to a chart recorder or to a signal averager. The change in delayed emission vs. illumination time, or induction, was measured by integrating the light emission between 0.5—5 ms. To avoid light-dark transients, the sample was usually preilluminated for 15 s before recording. All experiments were performed near 20°C unless otherwise stated.

The sample was illuminated with red light isolated from a tungsten-iodine lamp by a Corning filter (2—64). The light beam impinges onto the center of the cuvette with an incident intensity of $5.8 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. Plastic cuvettes (1 X 1 cm) were used as they do not show phosphorescence under our experimental conditions.

TSF-IIa samples were first diluted with unbuffered 1 M sucrose solution and used at a concentration of 10—20 μg chlorophyll in 4 ml. Chloroplasts were isolated from market spinach in 50 mM Tricine buffer, pH 7.5, containing 0.33 M sorbitol and 1 mM $MgCl_2$ and washed with the same buffer containing 10 mM KCl. In some cases, the sample was stored frozen and thawed immediately before use. Tris-washed chloroplasts [7] were prepared by incubating in 0.8 M Tris, pH 8.2, at 1 mg Chl/ml for 20 min at 0°C and then washed twice in 50 mM Tris buffer, pH 7.8, containing 0.4 M sucrose.

Results

In their earlier experiments, Vernon et al. [5] showed that the TSF-IIa particles emit delayed light, with an intensity approximately one-third that of chloroplasts on an equivalent chlorophyll basis, and with a much faster decay.

Effect of DCMU and ortho-phenanthroline

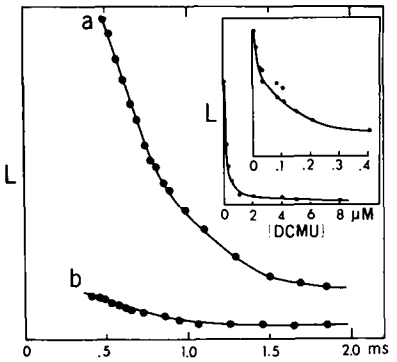
Fig. 1 shows typical decay kinetics of ms-delayed light of TSF-IIa particles in the absence (curve a) and in the presence (curve b) of 1 μM DCMU, measured within a time span of 0.3—2 ms after the termination of illumination. As shown by Vernon et al. [5] earlier, the decay of delayed emission is rapid and the addition of DCMU diminished the delayed-light intensity markedly.

The decay kinetics of delayed emission from TSF-IIa particles appear polyphasic. As illumination flashes are rather long and repetitive, it was difficult to estimate the decay rates from these measurements, but we made some semi-log plots for comparison purposes. These plots (not shown) yielded an initial phase of exponential decay with $t_{1/2}$ of 0.8—1.0 ms. Also the plot of the square root of delayed-light intensity vs. time did not yield a linear relationship in this time span.

As in chloroplasts, DCMU inhibited the ms delayed light emission markedly, even at very low DCMU concentrations. Fig. 1 inset shows the inhibition at different concentrations of DCMU measured at 0.5 ms after the termination of illumination. Low concentrations of DCMU (0.1 μM) seem to be effective in inhibiting the delayed emission in the TSF-IIa particles, although the exact concentration required to decrease the delayed emission by 50% varied among the different sample preparations. *o*-Phenanthroline also blocks electron flow at the level of the primary acceptor of Photosystem II and was a good inhibitor of delayed emission in TSF-IIa particles (data not shown).

Effect of artificial electron donors on the millisecond-delayed light emission

It was shown by Vernon and Shaw [8] that diphenylcarbazide (DPC) is an effective electron donor to Photosystem II for the reduction of DCIP. Fig. 2A shows that TSF-IIa particles in the presence of 0.5 mM DPC and pre-illumi-



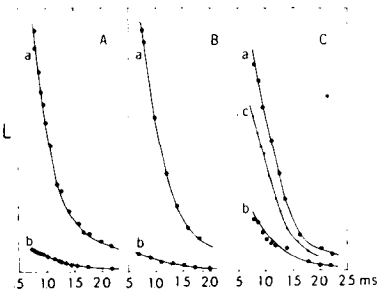


Fig. 1. Delayed light emission (intensity L in arbitrary units) from TSF-IIa particles without (a) and with (b) DCMU. The TSF-IIa particles were suspended in 50 mM TES-OH buffer, pH 7.3. Chl concentration, 13 μg in 2.5 ml. DCMU (1 μM) was added after illumination and decay of delayed light emission measured. Temperature was controlled at 18 ± 1°C. The excitation light was isolated through Corning 2-58 and 2-64 filters. The delayed light emission was recorded with a Biomation Model 610 transient recorder and a high chopping rate was used to measure the delayed emission in the short time range shown here. Inset: delayed light intensity plotted as a function of DCMU concentration. The delayed light intensity (L) was measured 0.5 ms after illumination. The smaller inset is a more detailed plot for the low DCMU concentration range. Other measurement conditions are the same as above.

Fig. 2. Effect of various secondary electron donors on the delayed light emission of TSF-IIa. (A) a, control; b, sample containing 0.5 mM DPC. (B) a, control; b, sample containing 2 mM ascorbate. (C) a, control; b, sample containing 0.5 μM $MnSO_4$; c, the sample in "b" stored in the dark for 1 min.

nated for 14—20 s yielded considerably lower delayed light emission. However, semi-log plots of the data showed no change in the decay kinetics of delayed emission on the addition of DPC. It was also observed that low concentrations of DPC (≈50 μM) does not affect the delayed-light intensity; but it is effective at 0.1 mM or higher.

Similar to DPC, ascorbate (at a relatively high concentration) (and also phenylenediamine, data not shown) also inhibit the delayed light emission, as shown in Fig. 2B. It was further observed that the addition of a trace amount of dithionite compltely abolishes the delayed emission in the ms range both in TSF-IIa particles and in isolated chloroplasts.

Mn^{2+} is known to be an effective electron donor to Photosystem II [9,10]; similarly, Mn^{2+} lowered the delayed light intensity (Fig. 2C curves a and b). If, however, the sample was allowed to stand for 1 min in the dark, the delayed light intensity increased momentarily (Fig. 2C curve c). Addition of the electron acceptor DCIP to the sample containing Mn^{2+} brings about an enhancement of the suppressed delayed emission signal, but the delayed light intensity was lower than in the absence of any donors. The lowering of delayed light intensity by Mn^{2+} seems to be limited to the initial 2 ms after illumination; the level of the slower-decaying components does not seem to be affected significantly. Again, the presence of Mn^{2+} does not appear to change the decay characteristics but only the amplitude of delayed light emission.

Similarly, addition of dimethyl benzoquinone to TSF-IIa particles containing Mn^{2+} also partially restored the level of delayed light intensity. As expected, addition of dimethyl benzoquinone to the sample treated with DCMU does not restore the suppressed delayed light level.

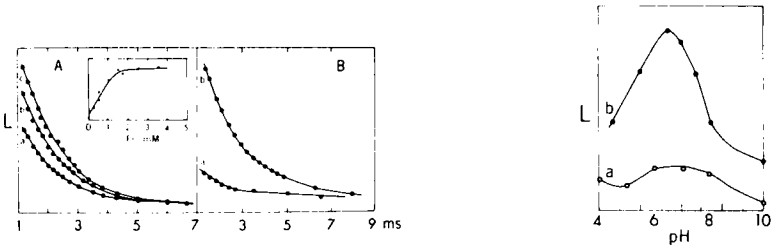


Fig. 3. Effect of secondary electron acceptors on the delayed light emission of TSF-IIa particles. (A) Delayed light emission at 50 (a), 125 (b) and 250 μM (c) ferricyanide. Inset: Delayed-light intensity plotted vs. ferricyanide concentration. (B) Delayed emission of TSF-IIa particles without (a) and with (b) 5 μM DCIP.

Fig. 4. pH dependence of the delayed light intensity (L) from TSF-IIa particles measured 0.8—1.0 msec from centers of illuminating flashes (i.e., peak luminescence intensity). The pH was varied by using MES, Tricine, TES, and glycine buffers, all at 10 mM. Curve a for sample alone; curve b for sample containing 0.5 mM ferricyanide.

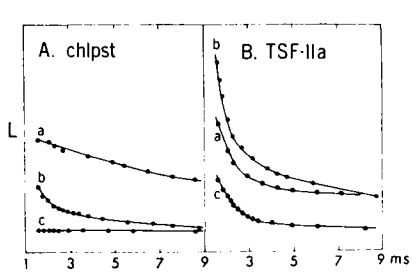
Effect of artificial electron acceptors on the millisecond-delayed light emission

Mayne [11] showed that the electron acceptors ferricyanide and pyocyanine increased the level of delayed light emission (at 3.7 ms) of chloroplasts over a wide range of concentrations. Similarly, Bertsch et al. [12] showed that at 1 ms after repetitive exciting flashes the secondary electron acceptors enhance the delayed light intensity and accelerate the decay in isolated chloroplasts. Fig. 3A shows that the addition of different amounts of ferricyanide enhances the delayed light intensity measured at ≈1 ms after the termination of illumination, and that the effect levels off at a ferricyanide concentration of 0.25 mM. As revealed by separate semi-log plots, it appears that the addition of different amounts of ferricyanide does not change the decay kinetics of delayed light emission.

Oxidized DCIP at 5 μM gives similar results (Fig. 3B). Stimulation of delayed emission by ferricyanide, DCIP and dimethyl benzoquinone was observed with almost all TSF-IIa preparations,, although the extent of stimulation varied and decreased with sample storage time. Addition of DCMU to the ferricyanide-treated samples abolished the delayed light emission signal, indicating that ferricyanide acts as an acceptor of electrons from the reduced endogenous acceptor of Photosystem II.

Fig. 4 shows the pH profile of delayed light emission measured 0.8 ms after the termination of illumination in the absence and in the presence of ferricyanide. The pH optimum is around 6.5—7.0; it is very similar to the pH optimum for electron transport reactions catalyzed by the same particles [8]. It is of interest to note that TSF-IIa particles emit delayed light at alkaline pH 9 and 10, and even show stimulation at this pH by artificial electron acceptors.

Felker et al. [13] showed that the plastoquinone antagonist 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB), which blocks electron flow after the plastoquinone pool, does not inhibit the ms-delayed emission but under some conditions increases the delayed light intensity, in contrast to the case of DCMU, which blocks electron flow to plstoquinone. Fig. 5A shows the results



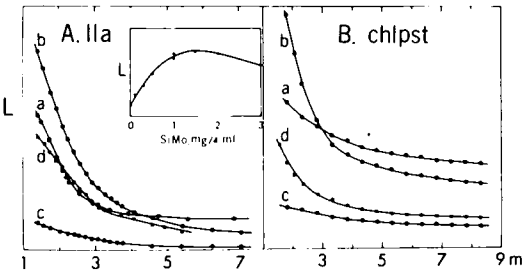


Fig. 5. Effect of DBMIB on the ms-delayed light emission from chloroplasts and TSF-IIa particles. (A) Spinach chloroplasts suspended in 50 mM phosphate buffer, pH 7.6; chlorophyll concentration: 28 μg/4 ml. DBMIB dissolved in ethanol/ethylene glycol (1 : 1). Samples containing no addition (a), 5 μM DBMIB (b), and 1 μm DCMU (c). (B) TSF-IIa particles at Chl concentration of 20 μg/4 ml. Samples containing no addition (a), 1 μM DBMIB (b) and 5 μM DBMIB (c).

Fig. 6. Effect of silicomolybdate on the ms-delayed light emission from TSF-IIa particles and chloroplasts. Silicomolybdate dissolved in dimethylsulfoxide and added to samples suspended in 4 ml 50 mM TES, pH 7.4. (A) TSF-IIa samples containing no addition (a), 100 μg silicomolygdate in 4 ml (b); 1 μM DCMU (c); and DCMU plus 50 μg SiMo in 4 ml (d). Inset: Delayed light intensity plotted vs. concentration of silicomolybdate. (B) Spinach chloroplasts suspended in 50 mM TES buffer, pH 7.4. Samples containing no addition (a); 100 μg silicomolybdate in 4 ml (b); 5 μM DCMU (c); DCMU plus silicomolybdate (d).

obtained with isolated spinach chloroplasts, which essentially agree with the results of Felker et al. [13]. DBMIB at low concentrations (up to 2.5 μM) induced a stimulation of delayed emission in TSF-IIa particles (Fig. 5B), although a high concentration of DBMIB (5 μM) decreased the delayed light intensity.

The addition of ferricyanide to the TSF-IIa sample containing 2.5 μM DBMIB did not cause any further enahncement, but the addition of DCMU to the DBMIB-treated TSF-IIa sample inhibited the delayed light emission.

Effect of silicomolybdate in the absence and in the presence of DCMU

It has been shown that silicotungstate [14] and silicomolybdate [15] can bring about a DCMU-insensitive Hill reaction in chloroplasts, which suggests that these compounds act as electron acceptors very close to the endogenous primary acceptor Q of Photosystem II. Zilinskas and Govindjee [16] have shown silicomolybdate to inhibit the chlorophyll *a* fluorescence of isolated chloroplasts as well as delayed light emission measured seconds after interruption of the excitation light.

We have investigated the effect of silicomolybdate on the delayed light emission of TSF-IIa particles and isolated chloroplasts in the presence and absence of DCMU. Fig. 6A shows that the addition of silicomolybdate brings about a large stimulation of ms-delayed emission of the TSF-IIa particles. A comparison of semi-log plots of the decay of delayed emission with time indicates that silicomolybdate apparently does not alter the decay kinetics to any large extent, but rather causes a large stimulation of the delayed light intensity. The extent of stimulation depended on the amount of silicomolybdate added (Fig. 6A, inset). Although the optimum silicomolybdate concentration varied somewhat with the TSF-IIa preparations, maximum stimulation of ms-delayed

light intensity was observed at concentrations ranging from 25 to 50 μg/ml. Silicomolybdate at high concentrations, however, lower the delayed light emission. Fig. 6A also shows that addition of silicomolybdate to samples containing DCMU completely relieves the inhibition of delayed light emission by the latter.

Fig. 6B shows the stimulatory effect of silicomolybdate on the delayed light intensity in broken spinach chloroplasts. Addition of silicomolybdate either dissolved in dimethylsulfoxide or in water induces a large initial increase in delayed emission and also an accelerated decay kinetics, causing a cross-over in the intensity curves of certain samples at about 3 ms after illumination. Addition of silicomolybdate to DCMU-treated chloroplasts partially restored the ms-delayed light emission; the extent of restoration is not as high as in TSF-IIa particles.

With both isolated chloroplasts and TSF-IIa particles, the addition of ferricyanide to samples already containing silicomolybdate caused no increase in the extent of delayed-light intensity, but rather a slight suppression was usually observed.

Effect of various "uncouplers" on the delayed light emission of TSF-IIa particles

Addition of CCCP or FCCP to the TSF-IIa particles containing silicomolybdate lowers the amplitude of the delayed emission signal, with FCCP more inhibitory than CCCP, as expected (Fig. 7A). Both CCCP and FCCP lowered the delayed light intensity in TSF-IIa with or without any acceptor such as ferricyanide or silicomolybdate present. Antimycin A, which binds to *b*-type cytochromes, also lowered the intensity of delayed emission in TSF-IIa particles (Fig. 7B). On the other hand, NH_4Cl seems to be ineffective (Fig. 7C), or in some samples caused a slight stimulation of the ms-delayed emission. The action of CCCP and FCCP on delayed light emission is probably due to an inhibition of Photosystem II on the oxidizing side [17] (see below).

Temperature dependence

It has been shown previously [3] that heat treatment impairs the photochemical activity of the TSF-IIa particles as measured by the DPC-supported

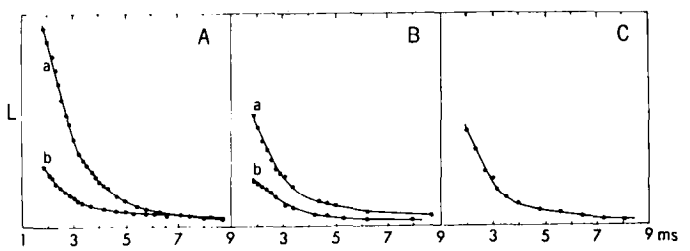
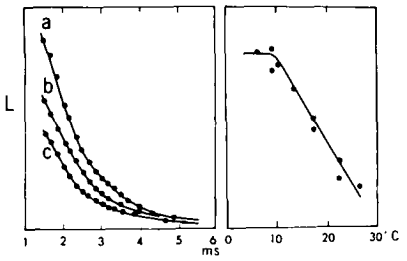


Fig. 7. Effect of FCCP, antimycin A and NH_4Cl on the delayed light emission of TSF-IIa particles. (A) TSF-IIa particles suspended in 50 mM Tricine buffer, pH 8.0, and containing silicomolybdate (a) or silicomolybdate plus 0.1 μM FCCP (b). (B) Sample containing 10 μM ferricyanide (a) or additional 5 μM antimycin A (b). (C) Sample without or with 25 mM NH_4Cl.



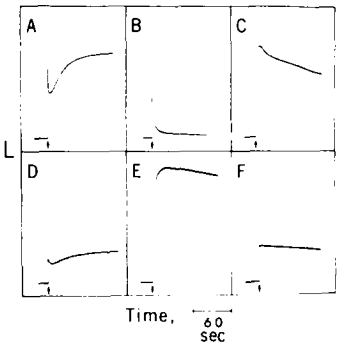


Fig. 8. Effect of temperature on the ms-delayed light emission of TSF-IIa particles. Samples were suspended in TES buffer at a chlorophyll concentration of 13 μg in 4 ml; the sample also contained 0.5 mM ferricyanide. Left: (a) 10°C, (b) 16°C, and (c) 22°C. Right: Delayed light emission intensity plotted vs. temperature.

Fig. 9. Induction changes of the delayed light intensity in chloroplasts (top row) and TSF-IIa particles (bottom row). Steady-state delayed light intensity was measured between 0.5 and 5 ms after illumination. Chloroplasts were suspended in phosphate buffer, at a Chl concentration of 35 μg in 4 ml. Chloroplast samples with no addition (a), 2 μM DCMU (B), or 0.5 mM ferricyanide (C). TSF-IIa particles with no addition (D), 0.5 mM ferricyanide (E) or 0.1 mM NH_4Cl (F).

DCIP reduction activity. The delayed light emission is almost abolished in samples which have been incubated for 5 min at temperatures of 40°C or above. Fig. 8 shows that the delayed-light intensity decreased as the temperature of the sample was increased.

Induction of millisecond-delayed light emission

The delayed-light intensity measured in chloroplasts as an integrated signal between 0.5 and 5 ms following illumination shows a typical signal as shown in Fig. 9A. Addition of DCMU abolished the slow increase of the induction curve (Fig. 9B). Addition of ferricyanide brought about a rapid rise and then a slower rise followed by a monotonous decline of the signal with illumination time (Fig. 9C). NH_4Cl inhibited the ms-delayed emission and suppressed the induction changes (not shown).

TSF-IIa particles exhibit somewhat similar induction patterns without any addition of electron acceptors such as ferricyanide (Fig. 9D). Addition of ferricyanide increased the level of delayed light emission and produced a strikingly similar induction change as seen in chloroplasts (Fig. 9E). However, addition of NH_4Cl did not inhibit the delayed emission intensity as it did in chloroplasts (Fig. 9F). The induction pattern of delayed light emission in TSF-IIa particles is also dependent on the pH of the suspending medium.

Discussion

Delayed light emission is predominantly a characteristic property of Photosystem II, although in recent years delayed light emission from Photosystem I has also been observed [18]. The magnitude of delayed emission originating from Photosystem I is much smaller than from Photosystem II [19]. The

results presented in this report lend further support to the Photosystem II nature of the TSF-IIa particles isolated from spinach by Triton treatment.

Although the TSF-IIa particles are enriched in Photosystem II reaction centers [3], the intensity of delayed light emission, as initially reported by Vernon et al. [5], is lower than in native chloroplasts. This can be understood from the fact that the chloroplasts exhibit slower decay kinetics in contrast to more rapid decay kinetics observed in the TSF-IIa particles. Secondly, the delayed light emission is emitted from the antenna chlorophyll *a* molecules that are in the vicinity of the reaction center chlorophyll (*P*-680) and probably not from the reaction centers themselves. Thus it seems likely that in the small TSF-IIa particles there would be less probability of re-exciting an antenna chlorophyll *a* molecule than in the native chloroplasts. The dependence of delayed light emission of TSF-IIa particles on excitation intensity (not shown) shows an almost linear rise of signal amplitude with increasing excitation intensity, which is in contrast to chloroplasts where delayed-light intensity increases almost hyperbolically at low excitation intensities [20,21].

The rapidly decaying kinetics of delayed emission seen in the TSF-IIa particles are similar to those of Tris-washed chloroplasts, namely, the decay of delayed emission becomes accelerated, compared with that in untreated chloroplasts. Addition of electron donors lowers the delayed light intensity, and this is again stimulated when acceptors such as DCIP or ferricyanide are added. It is of interest to note that the addition of electron acceptors without the addition of any exogenous electron donors does not bring about an increase of delayed emission at 1 ms in Tris-washed chloroplasts. On the other hand, these electron acceptors cause an enhancement of the ms-delayed light intensity in the TSF-IIa particles. It has been shown that Tris treatment completely blocks electron flow to the primary donor of Photosystem II [23]. Thus, it seems reasonable to conclude that TSF-IIa particles, unlike Tris-washed chloroplasts, contain some endogenous donor(s). This assumption has received support from the results obtained earlier in this laboratory [2] that these particles can photoreduce cytochrome *b*-559 at room temperature without the addition of any exogenous electron donor such as DPC.

Thus, our results on delayed light emission in the TSF-IIa particles may be interpreted with the basic model of Lavorel [24] that delayed light emission is a direct expression of a radiative back reaction of the primary photoact. Denoting the Photosystem II primary donor (*P*-680) by *P* and the primary acceptor by Q, one may formulate the photochemical charge separation and the recombination giving rise to delayed light emission as $P \cdot Q + h\nu \rightleftharpoons P^* \cdot Q \rightleftharpoons P^+ \cdot Q^-$, proceeding in the forward and reverse directions. Presumably the excitation on the primary donor can migrate back through the antenna chlorophyll molecules, leading to delayed light emission. The ms-delayed emission measured with a phosphoroscope originates from the $Z^+ \cdot P^+ \cdot Q^-$ state when the physiological donor of Photosystem II designated as Z is in the oxidized state. The intensity of delayed emission in this time range will depend on the concentration of open traps, namely, $Z^+ \cdot P \cdot Q$, before photoactivation, and indirectly on the momentary intervention of secondary electron donors and acceptors with the activated primary components. As the reduction of P^+ by Z is very fast, the concentration of $Z^+ \cdot P \cdot Q$ will depend on the equilibrium between

$D \cdot Z^+$ and $D^+ \cdot Z$, on the one hand, where D is the secondary donor that reduces Z^+, and, on the other hand, the reoxidation of Q^- by the A-pool (i.e., $Q^- \cdot A \rightarrow Q \cdot A^-$) [25]. Mayne [11] and Bertsch et al. [12] have already shown that both increased intensity and decay rate of the ms-delayed emission in chloroplasts are brought about by the addition of secondary electron acceptors which facilitates rapid reoxidation of Q^-. In the TSF-IIa particles, the plastoquinone pool is present in limited amounts [1,26], and this limitation hinders reoxidation of Q^-, and thus addition of electron acceptors like ferricyanide increases the ms-delayed light intensity mainly due to regeneration of $Z^+ \cdot P \cdot Q$.

We have also observed that DBMIB does not inhibit DPC-supported DCIP reduction in the TSF-IIa particles, which again suggests a lack of plastoquinone pool in these preparations. As shown in Fig. 5B, DBMIB acts as an effective electron acceptor and enhances the delayed light emission in these particles. DBMIB was found to be much more effective than some quinone-type Hill oxidants such as dimethyl benzoquinone.

Addition of electron donors such as DPC alone would likely keep Z^+ in the reduced state and this would in turn tend to keep the reaction center in the $Z \cdot P \cdot Q^-$ state. Thus, the addition of electron donors or other reductants such as ascorbate or even dithionite caused a lowering of ms-delayed light emission in the TSF-IIa particles.

We have observed (data not shown) that the dark decay of fluoresence yield in these particles, after cessation of illumination, is much slower in the presence of exogenous electron donors than without them, which suggests that addition of exogenous donors tends to keep the reaction centers in the $Z \cdot P \cdot Q^-$ state.

Mn^{2+} was found to be a very effective electron donor to the TSF-IIa particles. As the decay kinetics of delayed light emission in the presence of DPC are similar to those when Mn^{2+} are present, it appears that these two donors feed electrons at the same site. Recently, however, Babcock and Sauer [27] have presented evidence that Mn^{2+} feeds electrons directly to $P\text{-}680^+$ and other donors to Z^+.

Delayed light emission in the TSF-IIa particles was found to be very sensitive to DCMU (see Fig. 1) or *o*-phenanthroline. As DCMU or *o*-phenanthroline blocks electron flow from Q^-, the long illuminating flashes would tend to generate $Z^+ \cdot P \cdot Q^-$ states. Addition of DCMU in the presence of an electron donor causes further decrease of the ms-delayed light emission because of predominance of closed traps in the form of $Z \cdot P \cdot Q^-$. This situation is similar to the addition of NH_2OH to isolated chloroplasts [28] or agal cells [29] in the presence of DCMU, where the back reaction is totally inhibited due to an accumulation of the $Z \cdot P \cdot Q^-$ states.

It is of interest to note that silicomolybdate acts as an effective acceptor in relieving the DCMU inhibition both in isolated chloroplasts and in TSF-IIa particles. The restoration of ms-delayed emission occurs whether DCMU is added first, or when the sample is illuminated and then silicomolybdate added, or when silicomolybdate and DCMU are added simultaneously to the TSF-IIa particles. These data strongly suggest that the site of electron acceptance by silicomolybdate is close to Q^- as DCMU blocks electron flow at the level of Q^-. The inhibition of delayed light emission seen by Zilinskas and Govindjee [16] in isolated chloroplasts most likely relates to a cross-over in the decay kinetics as seen in Fig. 6.

The inhibition observed with the uncouplers CCCP and FCCP is likely to be due to accelerated deactivation of Z^+ [30] or to electron donation-type side effects [31] that are known to be associated with these compounds. As expected, NH_4Cl did not show any effect on the delayed emission in TSF-IIa particles. The modification of ms-delayed emission in chloroplasts by uncouplers has been characterized by Mayne [11] and Bell et al. [17]. The inhibition of delayed light emission in the TSF-IIa particles by CCCP and FCCP is probably due to the action of these compounds on the oxidizing side of Photosystem II [17] rather than the inhibition by uncouplers as reported by Mayne [11], since according to the presently accepted theory of photophosphorylation one would not expect simple particles, as contrasted to vesicles, to be affected by uncouplers. The lack of effect of NH_4Cl is consistent with the non-vesicular nature of these particles.

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References

1 Vernon, L.P., Shaw, E.R., Ogawa, T. and Raveed, D. (1971) Photochem. Photobiol. 14, 343—357
2 Ke, B., Vernon, L.P. and Chaney, T. (1972) Biochim. Biophys. Acta 256, 345—357
3 Ke, B., Sahu, S., Shaw, E.R. and Beinert, H. (1974) Biochim. Biophys. Acta 347, 36—48
4 Ke, B. and Shaw, E.R. (1972) Biochim. Biophys. Acta 275, 192—198
5 Vernon, L.P., Klein, S., White, F.G., Shaw, E.R. and Mayne, B.C. (1971) Proc. 2nd Int. Congr. Photosyn. Stresa (Forti, G., Avron, M. and Melandri, A., eds.), pp. 801—812, Dr. W. Junk Publ., The Hague
6 Clayton, R.K. (1965) J. Gen. Physiol. 48, 633—646
7 Yamashita, T. and Butler, W.L. (1968) Plant Physiol. 43, 1978—1986
8 Vernon, L.P. and Shaw, E.R. (1969) Plant Physiol. 44, 1645—1649
9 Izawa, S. (1970) Biochim. Biophys. Acta 197, 328—331
10 Ben-Hayim, G. and Avron, M. (1970) Biochim. Biophys. Acta 205, 86—94
11 Mayne, B.C. (1967) Photochem. Photobiol. 6, 189—197
12 Bertsch, W., West, J. and Hill, R. (1969) Biochim. Biophys. Acta 172, 525—538
13 Felker, P., Izawa, S., Good, N.E. and Haug, A. (1973) Biochim. Biophys. Acta 325, 193—196
14 Girault, G. and Galmiche, J.M. (1974) Biochim. Biophys. Acta 333, 314—319
15 Giaquinta, R.T., Dilley, R.A., Crane, F.L. and Barr, R. (1974) Biochem. Biophys. Res. Commun. 59, 985—991
16 Zilinskas, B.A. and Govindjee (1975) Biochim. Biophys. Acta 387, 306—319
17 Bell, D.H., Haug, A. and Good, N.E. (1978) Biochim. Biophys. Acta 504, 446—455
18 Shuvalov, V.A., Klimov, V.V. and Krasnovsky, A.A. (1976) Mol. Biol. 10, 326—337
19 Matorin, D.N., Venediktov, P.S., Gashimov, R.M. and Rubin, A.B. (1976) Photosynthetica 10, 266—273
20 Strehler, B.L. and Arnold, W. (1951) J. Gen. Physiol. 34, 809—820
21 Goedheer, C. (1962) Biochim. Biophys. Acta 64, 294—308
22 Bertsch, W.F. and Lurie, S. (1971) Photochem. Photobiol. 14, 251—276
23 Mohanty, P., Braun, B.Z. and Govindjee (1972) FEBS Lett. 20, 273—276
24 Lavorel, J. (1976) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 223—317, Academic Press, New York
25 Van Gorkom, H.J. and Donze, M. (1973) Photochem. Photobiol. 17, 333—342
26 Ke, B., Hawkridge, F.M. and Sahu, S. (1976) Proc. Natl. Acad. Sci. U.S. 73, 2211—2215
27 Babcock, G.T. and Sauer, K. (1975) Biochim. Biophys. Acta 396, 48—62
28 Bennoun, P. (1970) Biochim. Biophys. Acta 216, 357—363
29 Mohanty, P., Mar, T. and Govindjee (1971) Biochim. Biophys. Acta 253, 213—221
30 Renger, G. (1973) Biochim. Biophys. Acta 314, 113—116
31 Homann, P. (1971) Biochim. Biophys. Acta 245, 129—143