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DISCLAIMER

Electron Acceptors Associated With P700 in Triton Solubilized Photosystem I Particles From Spinach Chloroplasts

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Abstract

Flash-induced absorption changes of Triton-solubilized Photosystem 1 particles from spinach were studied under reducing and/or illumination conditions that serve to alter the state of bound electron acceptors. By monitoring the decay of P700 following each of a train of flashes, we found that P430 or components resembling it can hold 2 equivalents of electrons transferred upon successive illuminations. This requires the presence of a good electron donor, reduced phenazine methosulfate or neutral red, otherwise the back reaction of $P700^{+}$ with P430 occurs in about 30 ms. If the two P430 sites, designated Centers A and B, are first reduced by preilluminating flashes or chemically by dithionite under anaerobic conditions, then subsequent laser flashes generate a 250 μs back reaction of $P700^{+}$, which we associate with a more primary electron acceptor A_2. In turn, when A_2 is reduced by background (continuous) illumination in presence of neutral red and under strongly reducing conditions, laser flashes then produce a much faster (3 μs) back reaction at wavelengths characteristic of P700. We associate this with another more primary electron acceptor, A_1, which functions very close to P700. The organization of

these components probably corresponds to the sequence $P700 \cdot A_1 \cdot A_2$ P430 $\begin{bmatrix} A \\ B \end{bmatrix}$. The relation of the optical components to acceptor species detected by EPR, by electron-spin polarization or in terms of peptide components of Photosystem 1 is discussed.

Preliminary experiments with broken chloroplasts suggest that an analogous situation occurs there, as well.

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Abbreviations: PMS, phenazine methosulfate; DMT, dimethyl triquat, 1,1'-trimethylene-4,4'-dimethyl-2,2'-bipyridylium bromide; $DCIPH_2$, 2,6-dichlorophenolindophenol, reduced.

INTRODUCTION

The light reaction of Photosystem 1 of higher plants involves the transfer of an electron from the P700 chlorophyll of the reaction center to one or more bound electron acceptors. These reactions can be followed either optically using sensitive difference spectroscopy [1-3] or by electron paramagnetic resonance to detect the species containing unpaired electrons [4-6]. For P700 there is good agreement between the two methods for experiments carried out at room temperature [7]. For the electron acceptors the situation is more complex. Optical signals observed in the blue at room temperature have been used to characterize the species P430, [3,8] which appears to correlate with a low temperature EPR signal characteristic of an iron-sulfur center [9] that has been designated Center A [10,11]. Additional low temperature EPR signals from iron-sulfur Center B [10,11] and from a species designated X have been reported [11-14], and they are also candidates for participation on the acceptor side of Photosystem 1. Electron spin polarization studies show that, in the early stages of the photochemistry, there is coupling between the unpaired electron on $P700^+$ and that on the counter radical, probably X^- [15].

We have studied the kinetics of flash photo-induced optical changes of Triton-solubilized subchloroplast particles enriched in Photosystem 1 [16]. These particles appear to contain P700 together with the bound electron acceptors; but the normal electron donors, plastocyanin or cytochrome f, are not functional [17]. By subjecting these particles to various regimes involving reducing conditions and background illumination, we have been able to use the kinetics of re-reduction of $P700^+$ following a flash to monitor the state of the electron acceptors and to determine something about their sequence [18]. In addition to demonstrating the occurrence of two intermediate species, presumably low potential electron acceptors, between P700 and P430, we find that acceptors at the level of P430 are able to accept two electrons following successive saturating flashes that turn over P700 twice. We interpret these results in terms of recent studies of low temperature EPR and of electron spin polarization.

MATERIALS AND METHODS

Spinach chloroplasts were prepared from leaves grown in a greenhouse and were isolated in sucrose (0.4M), KCl (10 mM), $MgCl_2$ (2 mM) and tricine buffer (50 mM, pH 7.6). Subchloroplast particles (TSF1) solubilized with Triton X-100 were prepared according to the procedure of Vernon and Shaw [16] and were stored at -20°C until needed.

Neutral red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) or PMS (phenazine methosulfate) was dissolved in water at 2mM and 1mM, respectively. Dimethyl triquat (DMT; 1,1'-trimethylene-4,4'-dimethyl-2,2'-bipyridilium bromide) was a gift generously provided by Imperial Chemical Industries, Ltd., Bracknell, Berkshire, England.

Reaction mixtures were prepared by first thawing a portion of the TSF1 preparation and homogenizing in the appropriate buffer and added reagents (except dithionite). For anaerobic experiments the reaction mixture was then frozen and degassed under vacuum 3 times in an evacuable cuvette (1 cm path) with two side arms. After the degassing, solid dithionite was added from one of the side arms and the sample was kept in darkness until the start of the experiment. Anaerobic experiments where dithionite was included were carried out in glycine buffer (0.2 M, pH 10), but when ascorbate or aerobic dithionite was used as reducing agent the buffer was tricine (0.02 M, pH 7.6).

The experiments were carried out using a rapid-transient spectrometer system that is capable of resolving components to about 1 μs duration.

The light for the measuring beam and for background illumination, when present, came from a quartz tungsten-iodine incandescent lamp. The radiation was filtered either by an interference filter (bandwith 2 nm at half maximum) or Wratten 87 gelatin filter prior to the sample when no background illumination was desired or by an RG630 filter (Schott) that passed red light to provide a background. The light transmitted by the sample was focused on the entrance slit of a Bausch and Lomb grating monochromator (500 mm) with 7 nm bandpass and, in most cases, a supplementary interference filter (2-3 nm bandpass). Light was detected using a silicon photodiode PIN-10 (UDT, Santa Monica, CA) for absorption changes in the red or near IR. Signals from the detector were recorded using a Transient Digitizer (Tektronix R7912) coupled to a multichannel analyzer (Didac 4000, Intertechnique) where the signals from several experiments could be combined. Flash excitation of the sample was provided at 90° to the measuring beam using either a Q-switched ruby laser (Quantel, France; λ, 694 nm, 10 ns duration) or a flash-lamp pumped dye laser (ElectroPhotonics, Belfast; 605 nm, 1 μs duration). In experiments involving a train of flashes spaced 30 ms apart, the preilluminating flashes were obtained from a "Stroboslave" xenon flash unit (General Radio) and the final flash from the dye laser. For measurements in the blue, the monochromator was inserted between the lamp and the cuvette, and the measuring light was detected with a photomultiplier whose output, after amplification, was fed directly into a multi-channel analyzer. The cuvette was excited by two synchronized "Stroboslave" xenon flash lamps, filtered by RG 630 and Calflex (IR absorbing) filters. A 4-96 filter (Corning) supplemented by either a Wratten 44A (Kodak) or a BG 12 (Schott) filter were used in front of the photomultiplier.

For each set of experimental transient signals recorded, an equal number of flash artifacts with the measuring beam blocked was subtracted.

All experiments were performed at room temperature (21 C) in a square (1 cm x 1 cm) cuvette.

RESULTS

To characterize our TSF 1 particle with respect to Photosystem 1 reactions, we carried out initial experiments under conditions analogous to those used by Hiyama and Ke [3,19] in defining the relation between P700 and P430. In the presence of ascorbate and 2,6-dichlorophenolindophenol ($DCIPH_2$) as electron donor and benzyl viologen as acceptor we observed flash-induced absorption changes at 703 or 820 nm that reversed slowly, during several seconds, in an aerobic sample. The spectrum of these changes between 370 nm and 500 nm and between 703 nm and 820 nm is that of P700, reported previously [3]. In particular, we observed isosbestic wavelengths at about 408, 445 and 725 nm.

In the absence of benzyl viologen and under anaerobic conditions, a sample of TSF1 particles containing ascorbate and $DCIPH_2$ exhibits flash-induced absorption changes at 703 or 820 nm that reverse more rapidly, but with a biphasic decay. About 75% of the reversal occurs with a 30 ms halftime; the remainder is much slower. The faster component is characteristic of the back reaction between $P700^+$ and $P430^-$, and the difference spectrum that we observe in the red and the blue is similar to that reported by Ke [3]. We find essentially identical behavior under aerobic or anaerobic conditions. We are not able to detect any faster components in the decay, to the instrument limit of about 1 μs, at 703 or 820 nm under these experimental conditions regardless of the presence of benzyl viologen.

Dithionite (Anaerobic). For samples of TSF1 particles suspended in alkaline buffer (glycine, 0.2 M, pH 10) and then degassed under vacuum, the subsequent addition of sodium dithionite (2 mg ml^{-1}) produces a more rapid relaxation at room temperature following flash excitation. Measurements at 703 or 820 nm are shown in Fig. 1. The back reaction under these conditions was analyzed using a semi-logarithmic plot of the data. It occurs almost entirely (> 90%) via a single exponential decay with a halftime of about 250 μs. The difference spectrum in the region from 675 to 900 nm (Fig. 2) closely resembles that of P700. Measurements in the blue region (385 to 600 nm) are also shown in Fig. 2. By contrast with P700 alone (solid curve), which exhibits positive absorbance changes between 395 and 400 and between 445 and 455 nm, the 250 μs decay component seen in the presence of dithionite has essentially zero amplitude in these wavelength regions. In this respect the difference spectrum resembles that of P700 + P430 [3]; however, the decay kinetics that we observe is about 100 times faster at room temperature and P430 is known to remain reduced in the dark under strongly reducing and anaerobic conditions. Immediately after mixing dithionite with the sample in darkness, the decay of P^{+}_{700} is slow. The 250 μs decay is observed only after the sample has received at least 10 flashes, spaced by about 15s.

Neutral red + Background Illumination (Anaerobic). Shuvalov, et al. [20,21] reported that the addition of a good electron donor like neutral red, in the presence of dithionite at pH 8.0, leaves Photosystem 1 in the state $P700 \cdot P430^{-}$ at a time 2 ms following illumination. For a similar reaction mixture under anaerobic conditions we observe decay of $P700^{+}$ in the millisecond range following a laser flash (Fig. 3, left). If, however, a background of red light ($\lambda >$ 630 nm) is provided prior to and during the flash, then a large portion of the absorbance change at 820 nm reverses much more rapidly (Fig. 3, right). Using a faster sweep rate for the digitizer (Fig. 4) we are able to resolve a 3 μs decay component under these conditions. (The experiments shown in Fig. 4 were carried out with the added presence of dimethyl triquat, DMT, a low-potential electron transfer mediator that has been shown to facilitate P430 reduction [22]. We found identical behavior in our experiments in the absence of DMT). The spectrum of this absorption change resembles that of P700 in the region from 703 to 940 nm; however, there appears to be a shift in the isosbestic wavelength near 720 nm (Fig. 5). This is seen most clearly at 720 nm, where a negative transient absorption change in the absence of background illumination becomes positive when background light is added to the same reaction mixture. Furthermore, the major components of the pronounced biphasic decay seen with background illumination exhibit different relative magnitudes at different wavelengths throughout this spectral region.

After a series of experiments performed with background illumination, a flash given without background light leads to a recovery of the P700 absorption change in about 250 μs, as described above.

Upon introducing air to the anaerobic samples containing dithionite and neutral red, the rapid transients are not immediately quenched. By contrast with the behavior expected for triplet species, the decay gradually becomes slower (approaching 2-3 ms in the absence of background illumination) for the absorption transients in the long wavelength region as the sample becomes fully oxygenated following agitation under air. In one experiment with background illumination, the 3 μs transient was not immediately affected upon mixing air into the solution.

Phenazine Methosulfate (Aerobic) - Flash Series. To prevent the back reaction of $P700^+$ with $P430^-$ it is sufficient to add reduced PMS or neutral red to the TSF1 particles. At proper concentrations these reagents are capable of reducing $P700^+$ more rapidly than the back reaction. We find, for example, that a concentration of 30 μM PMS (reduced) is sufficient to decrease the decay time for the flash induced P700 transient to about 2.5 ms, compared with a 30 ms decay in the absence of PMS. Dithionite added to the sample at pH 7.6 under aerobic conditions serves to keep the PMS in the reduced form without also reducing the P430 chemically prior to illumination. By applying a train of saturating flashes spaced at 30 ms intervals, we are then able to monitor the capacity of the electron acceptor system to accept electrons, because the P700 becomes rapidly restored by the reduced PMS following the initial flash(es).

The response at 820 nm to individual saturating flashes in such a sequence is shown in Fig. 6. In this particular experiment the last flash only was monitored, and it was provided by the dye laser. The preceding flashes in the sequence were provided by a Stroboslave flash lamp. Other arrangements gave identical results. The response to a single flash after a dark period of several minutes is shown in the top curve. The decay occurs with a halflife of 2.4 ms, determined primarily by the PMS concentration. When the laser flash is the second flash in the sequence, as seen in the second trace from the top in Fig. 6, the decay at 820 nm is about the same (1.9 ms). With two preceding flashes, however, the response to the third flash is appreciably faster (0.32 ms) and it remains fast (0.26 ms) following a fourth flash (Fig. 6, lower traces). The latter times are, in fact, essentially the same as those reported above when P430 has been reduced chemically prior to an illuminating flash.

A similar response pattern following successive flashes occurs for the bleaching of absorption at 720 nm. There is usually a small (10%) decrease in initial amplitude of the response between the first and the fourth flashes; however, these may be due to our failure to record accurately the initial response of the fast decaying signal seen on the later flashes. The experiment involving a flash train can be repeated on the same sample when an interval of several minutes in the dark allows it to recover the initial state.

Chloroplasts. Preliminary experiments were carried out with broken spinach chloroplasts in the presence of reduced PMS (50 μM) as a donor and with dithionite plus dimethyltriquat (anaerobic) to maintain a low potential in tris buffer (0.2 M, pH 9.0). In the absence of background illumination the P700 absorption change following a laser flash exhibits a reversible decay with a halftime of about 200 μs, measured at 820 nm. The signal exhibits normal saturation with increasing flash excitation intensity. When background illumination is added, the decay becomes dramatically faster ($t_{1/2}$ = 3 ± 1.5 μs). The difference spectrum of this fast transient response between 690 and 860 nm shown in Fig. 7 closely resembles that of $P700/P700^{+}$, and the value of the absorption change at 703 nm corresponds to a change of about 1 P700 for 300 to 400 chlorophylls. Upon removing the background illumination the slower kinetics is restored. During the course of a series of such experiments involving about 60 flashes on a single sample, there is an irreversible loss of absorption by the chloroplasts of 10 to 20% measured at 678 nm. The flash-induced absorption transients at 695 nm and longer wavelengths appear to be fully reversible, however.

DISCUSSION

Absorption changes associated with Photosystem 1 light reactions have been assigned to P700 oxidation (bleaching at 700 and 430 nm; increases in absorption from 730 to 850, 450 to 550 and 300 to 400 nm) [3] and to P430 reduction (broad bleaching between 370 and 470 nm; maximum at 430 nm about one-fourth that of the P700 bleaching) [3,19]. Ke and coworkers have provided detailed studies of the kinetics and the correlation of these changes, under conditions where their restoration is caused either by non-cyclic electron flow, cyclic electron flow or an internal back reaction [3]. In the present research we report evidence for the existence on the acceptor side of PS1 of two additional components that can be distinguished on the basis of the kinetics of their interaction with $P700^+$ plus the observation that P430 apparently can hold two electron equivalents.

We have investigated the properties of Triton-solubilized Photosystem 1 particles from spinach (TSF1 particles) where the normal electron donors (plastocyanin, cytochrome f) and terminal acceptors (soluble ferredoxin, $NADP^+$) are either absent or disconnected [17]. TSF1 particles do contain active P700 and bound iron-sulfur proteins [23]. We investigated the properties of these particles using laser-pulse excitation under five distinct sets of conditions:

(1) <u>Ascorbate/$DCIPH_2$</u> + <u>Benzyl Viologen</u> (<u>Aerobic</u>). This provides a good electron acceptor, benzyl viologen, and a relatively inefficient electron donor, $DCIPH_2$, under mildly reducing conditions. $P430^-$ produced by illumination is reoxidized rapidly by the benzyl viologen, and $P700^+$ is reduced only slowly (several seconds) by $DCIPH_2$ following a flash. The absorption changes observed are characteristic of P700 alone. The process can be summarized by the equation

$$DCIPH_2 + P700{\cdot}P430 + BV \xrightarrow{h\nu} DCIPH_2 + P700^+{\cdot}P430 + BV^- \xrightarrow{\underline{ca}\ 2\ sec} DCIP + P700{\cdot}P430 + BV^- \quad (1)$$

The rate of reoxidation is a function of $DCIPH_2$ concentration.

(2) Ascorbate/$DCIPH_2$. In the absence of a suitable electron acceptor for $P430^-$, the back reaction with $P700^+$ is the dominant relaxation process following a flash. Under these conditions the difference spectrum is characteristic of P700 and P430 together, with a relaxation time of about 30 ms for both components

$$P700 \cdot P430 \xrightarrow{h\nu} P700^+ \cdot P430^- \tag{2}$$

$$P700^+ \cdot P430^- \longrightarrow P700 \cdot P430 \quad \text{(30 ms back reaction)}$$

The rate of relaxation is independent of $DCIPH_2$ concentrations. The conditions (1) and (2) were both characterized by Ke [3].

(3) Dithionite (Anaerobic, pH 10). At a sufficiently low potential the relaxation time for $P700^+$ reduction decreases to 250 μs. We intrepret this to mean that $P430^-$ accumulates following the first few flashes and its reoxidation is prevented. A midpoint potential of -530 mv for the reduction of P430 was reported by Lozier and Butler [24] and by Ke [23]. Under reducing conditions (dithionite, pH 10, anaerobic) the photooxidation of $P700^+$ indicates the reduction of a new intermediate electron acceptor, which we designate provisionally A_2 and which undergoes a back reaction with $P700^+$ in 250 μs. The equation representing this process is

$$P700 \cdot A_2 \cdot P430^- \xrightarrow{h\nu} P700^+ \cdot A_2^- \cdot P430^- \tag{3}$$

$$P700^+ \cdot A_2^- \cdot P430^- \longrightarrow P700 \cdot A_2 \cdot P430^- \quad \text{(250 μs back reaction)}$$

The difference spectrum associated with this reaction is clearly distinct from that of P700 in the blue region (Fig. 2). The contribution of A_2 in the blue is not very different from that of P430. It is possible that A_2 is the low potential intermediate implicated by the experiment of Ke *et al.* [22] or the species X characterized using low temperature EPR measurements [11-14]. It may be also responsible for the delayed fluorescence seen under reducing conditions as reported by Shuvalov, *et al*, [20,21] and discussed below. The clues that we have suggest that condition (3) does not involve the triplet state of chlorophyll, as proposed by Shuvalov [21]. Indeed, the 250 μs phase is not accelerated when O_2 is dissolved in the sample, nor does its spectrum present a large positive absroption around 460 nm as does triplet chlorophyll a [25].

(4) *Neutral Red (or PMS) + Background Illumination + Dithionite (Anaerobic, pH 10)*. Combining an efficient electron donor with illumination under reducing conditions produces a new and much faster (3 μs) decay component of $P700^+$ following a flash. We suppose that the good electron donors compete with the back reaction of (3) so that A_2 becomes progressively reduced by the background illumination prior to the laser flash. In this view, the 3 μs reaction reflects the recombination of $P700^+$ with a new intermediate species, which we designate A_1. This reaction is

$$\xrightarrow[\text{light}]{\text{Background}} P700 \cdot A_1 \cdot A_2^- \cdot P430^- \xrightarrow{h\nu} P700^+ \cdot A_1^- \cdot A_2^- \cdot P430^- \qquad (4)$$

3 μs back reaction ($P700^+ \cdot A_1^- \to P700 \cdot A_1$)

The identity of A_1 is unknown. The absorption changes in the long wavelength region (Fig. 5) are consistent with P700 participation; again, a triplet should show greater sensitivity to admission of air than we see but that possibility cannot yet be ruled out. If it is a triplet, then it must be closely related to P700, because the bleaching at 703 nm corresponds precisely to that of P700 and requires that the acceptors of P700 (P430, A_2) are reduced. Thus it would be more similar to the state P^R observed in bacterial reaction centers [26] than to a triplet state of antenna chlorophyll.

(5) Phenazine Methosulfate + Dithionite (Aerobic, pH 7.6). By adding sufficient reduced PMS to prevent the back reaction of $P700^+$ with $P430^-$ following a flash, it is possible to apply a series of closely spaced (30 ms) saturating flashes and determine the number of equivalents of bound electron acceptors, relative to P700, that lie beyond A_2. The results shown in Fig. 6 indicate that there are two equivalents, and we designate them as P430A and P430B. This study is summarized in the following reactions

First Flash

$$P700 \cdot P430 \begin{bmatrix} A \\ B \end{bmatrix} \xrightarrow{h\nu} P700^{+} \cdot P430 \begin{bmatrix} A \\ B \end{bmatrix}^{-} \xrightarrow[2.5\ \mathrm{ms}]{PMSH_2} P700 \cdot P430 \begin{bmatrix} A \\ B \end{bmatrix}^{-} + PMS$$

Second Flash

$$P700 \cdot P430 \begin{bmatrix} A \\ B \end{bmatrix}^{-} \xrightarrow{h\nu} P700^{+} \cdot P430 \begin{bmatrix} A \\ B \end{bmatrix}^{-}_{-} \xrightarrow[2.0\ \mathrm{ms}]{PMSH_2} P700 \cdot P430 \begin{bmatrix} A \\ B \end{bmatrix}^{-}_{-} + PMS$$

Third, fourth, etc. flashes

$$P700 \cdot A_2 \cdot P430 \begin{bmatrix} A^- \\ B^- \end{bmatrix} \xrightarrow{h\nu} P700^{+} \cdot A_2^{-} \cdot P430 \begin{bmatrix} A^- \\ B^- \end{bmatrix}$$

250 μs back reaction

Under aerobic conditions at pH 7.6 the dithionite serves to reduce the PMS but not the P430 prior to the flashes. At 30 μM concentration PMS reduces P700 in 2.0 to 2.5 ms, which is ten times faster than the back reaction with P430 (30 ms, as in condition (2)). The fact that this relaxation time is seen following each of the first two saturating flashes indicates that P700 has transferred two successive equivalents of electrons to acceptors at the P430 level. Following the third and fourth flashes of the series, the 250 μs decay time characteristic of the back reaction of $P700^+$ with A_2^- is seen. This is ten times faster than the donation by $PMSH_2$ to $P700^+$. Two obvious candidates for the P430-level acceptors are the iron-sulfur proteins designated Center A and Center B, as seen in low temperature EPR studies. It is not clear at present whether Center B is an intermediate component between A_2 and P430A or whether Center B becomes reduced only when Center A is already reduced. Kinetic EPR studies may be required to answer this question.

This expanded array of electron acceptor components is diagrammed in the model shown in Fig. 8. It is no coincidence that this model bears a striking similarity to one proposed by Bengis and Nelson [27] for the structural organization of the Photosystem 1 reaction center and acceptor complex on the basis of peptide composition. In fact, several lines of research can be brought together in the model proposed in Fig. 8.

The optical changes associated with P430 [8] and the EPR signals attributed to a bound ferredoxin or iron-sulfur protein [28] were proposed to represent "primary acceptors" of Photosystem 1. Subsequent research showed that these observations probably represent the same species [8], but at the same time it was discovered that there are at least two components exhibiting EPR signals resembling those of iron-sulfur proteins [29] and with midpoint potentials of about -540 and -590 mv, [10,30] designated Center A and Center B, respectively. Each apparently corresponds to a 1-electron change [10,23]. Although these EPR signals can be monitored only at temperatures close to that of liquid helium, illumination at 77 K or during cooling from room temperature is effective in generating them along with $P700^+$. Normally only Center A is photoreduced [28];

however, if Center A is already reduced by illumination in the presence of dithionite, then Center B can become photoreduced [31]. The similarity to the behavior under our condition (5) leads us to propose that these centers play a similar role at room temperature. Following a brief saturating flash P700 becomes oxidized and P430A becomes reduced.. In the presence of a good electron donor like $PMSH_2$, $P700^+$ becomes rereduced without Center A becoming reoxidized. On a second flash the electron transferred from P700 goes to Center B to reduce it; again, $P700^+$ returns to its reduced form via electron transfer from reduced PMS. These two one-electron transfer steps produce a state where both Center A and Center B are reduced, and P700 is restored to its active (reduced) state.

In low temperature experiments using Triton-solubilized particles enriched in Photosystem 1, McIntosh, et al, [12] found that illumination produced an irreversible reduction of both Centers A and B, but there remained a reversible component of the EPR signal of $P700^+$ at 6K. Associated with this was a signal from a new EPR component, designated X, which has distinctive g-value components (1.78, 1.88, 2.08) and presumably contains iron in some unknown environment [11,14]. No room temperature optical signals have been attributed to this species, although Shuvalov et al, [20,21] have investigated optical changes under strongly reducing conditions and recent experiments by Ke, et al, [22] at low potentials point to the occurrence of an electron acceptor between P700 and P430. We attribute this behavior to the species A_2^- which undergoes a back reaction with $P700^+$ in 250 μs when P430 is prereduced either chemically by dithionite (anaerobic) or by two preilluminating flashes in the presence of reduced PMS. If this assignment is correct, then the experiments of Ke et al, [22] suggest that A_2/A_2^- has a midpoint potential of about

-730 mV. Shuvalov [21] observes delayed fluorescence with about 500 μs halflife under similar conditions, and assigns this to a triplet state of chlorophyll. It would appear that his room temperature experiments could be accounted for as well by charge recombination involving an intermediate electron acceptor. As a sidelight, it is interesting to note the contrast between the P430 difference spectra reported by Shuvalov [21] and those of Hiyama and Ke [19]. The former paper shows P430 to have a double-peaked spectrum in the blue, to have appreciably more amplitude relative to the P700 spectrum in the same spectral region, and to exhibit bleaching to 500 nm and beyond. The differences may arise because under the conditions of Shuvalov's study (dithionite + neutral red and background illumination) both Centers A and B are involved in the "P430" change, whereas for the studies of Hiyama and Ke (response to single flashes) only Center A was photoreduced. If this is so, then the difference between these two spectra would represent the difference spectrum of Center B. This clearly needs further investigation.

Unfortunately it is difficult at present to correlate the EPR studies on X^-, carried out at low temperature, with the optical studies relevant to A_2^-, carried out at room temperature. The interpretation is complicated by the fact that we find evidence for an additional component, A_1^-, which appears to reside even closer to P700. On the basis of EPR spin polarization studies, Dismukes <u>et al</u>, [15] have proposed that X^- lies close to $P700^+$ and that these species constitute the radical pair of Photosystem 1 that gives rise to spin polarization. Although A_1^- is a good candidate for this radical counterion to $P700^+$, it is possible that the magnetic interaction with the next species, A_2^-, would be strong enough to generate the observed spin polarization.

The observation of a fast (3 μs) component of P700 absorption change relaxation in broken chloroplasts in the presence of $PMSH_2$ and dithionite (anaerobic) suggests that the fast component (4) is not an artifact introduced by the detergent treatment in preparing TSF1 particles. Of course, to compete against this back reaction under normal conditions leading to photosynthetic energy conversion, the forward reactions of electron transfer must be much faster. It appears from the studies of Ke[32] that the electron transfer to P430 occurs in a time less than 100 ns; however, that study may need to be reevaluated if the difference absorption spectra of A_2, P430A and P430B are all similar to one another.

CONCLUSION

On the basis of flash-induced absorption changes of P700 under ambient and under reducing conditions, we conclude that there is a set of four electron acceptors associated with Triton-solubilized Photosystem 1 particles. The species previously designated P430, probably attributable to an iron-sulfur protein, consists of two centers, A and B, each of which is capable of holding one electron. When both of these P430 centers are reduced prior to a flash, another acceptor A_2 can receive an electron from P700; and when A_2 is reduced prior to a flash, yet another acceptor A_1 appears to function. This chain of acceptors must serve to produce a substantial and very rapid separation of charge across the photosynthetic membrane.

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FIGURE LEGENDS

Fig. 1. Absorption transients at 703 nm (left) and 820 nm (right) induced by a ruby laser flash (20 ns duration) incident on a sample of TSF1 particles. Anaerobic reaction mixture contains sodium dithionite (2 mg ml^{-1}), Triton (0.015%), glycine (0.2M, pH 10) and TSF1 particles sufficient to give $A_{672nm} = 1.61\ cm^{-1}$. The vertical bars indicate ΔA at each wavelength. At 703 nm, $t_{\frac{1}{2}} = 270$ µs (96%); at 820 nm, $t_{\frac{1}{2}} = 245$ µs (90%); the remaining signal at each wavelength was a slower component. No background illumination; each curve is the average of two flash response signals.

Fig. 2. Difference spectra for flash-induced absorption changes of TSF1 particles from spinach. Points ● and dashed curves summarizes the wavelength dependence under anaerobic conditions in the presence of dithionite, as described in Fig. 1. Solid curve represents the difference spectrum of $P700/P700^+$ measured in the blue for TSF1 particles (aerobic) in presence of ascorbate/$DCIPH_2$ and with benzyl viologen as electron acceptor; conditions as described by Ke [3]. Amplitudes are presented as the absorbance change, ΔA, divided by the absorbance at the 672 nm maximum, A_{max}.

Fig. 3. Absorption transients at 820 nm induced by a ruby laser flash on a TSF1 sample without background illumination (left; Wratten 87 filter before sample) and with background illumination (right; no Wratten 87 filter). The vertical bars indicate Δ A at 820 nm. Anaerobic reaction mixtures contain sodium dithionite (2 mg ml^{-1}),

Triton (0.015%), neutral red (10 μM) and glycine (0.2 M, pH 10), Reaction mixture for the left trace contained dimethyltriquat (DMT 8 μM) and TSF1 particles sufficient to give $A_{672\ nm}$ = 1.43 cm^{-1}; for the right trace, no DMT was present and $A_{672\ nm}$ was 1.17cm^{-1}. Each curve is the average of two flash response signals.

Fig. 4. Absorption transients at 703 nm (left) and 820 nm (right) induced by a ruby laser flash on a sample of TSF1 particles under background illumination. Anaerobic mixture contains sodium dithionite (2 mg ml^{-1}), Triton (0.015%), neutral red (10 μM) dimethyltriquat (DMT, 8 μM), glycine (0.2 M, pH 10) and TSF1 particles sufficient to give $A_{672\ nm}$= 1.43 cm^{-1}. At 703 nm, $t_{1/2}$ = 2.7 μs (85%); at 820 nm, $t_{1/2}$ = 2.8 μs (68%); additional slower components at each wavelength. Each curve is the average of two flash response signals.

Fig. 5. Difference spectra for the flash-induced absorbance changes of TSF1 particles from spinach. Reaction mixture and conditions as in Fig. 3. Closed circles ●, with background illumination; open circles O, without background illumination. Ordinate as in Fig. 2.

Fig. 6. Absorption transients at 820 nm for TSF1 particles induced by a saturating dye laser flash (λ = 605 nm, 1.0 μs half width) preceded by zero to three saturating preillumination flashes (not shown in the traces) from Stroboslave xenon flashlamp; spacing 30 ms between flashes. Aerobic mixture contains dithionite (1 mg ml^{-1}), Triton (0.005%), phenazine methosulfate (PMS, 30 μM), tricine (0.02M, pH 7.6) and TSF1

particles sufficient to give $A_{673\ nm} = 1.11\ cm^{-1}$.

Top curve - laser flash only, $t_{1/2} = 2.4$ ms (> 95%)

Second curve - one preillumination flash; $t_{1/2} = 1.9$ ms (> 95%)

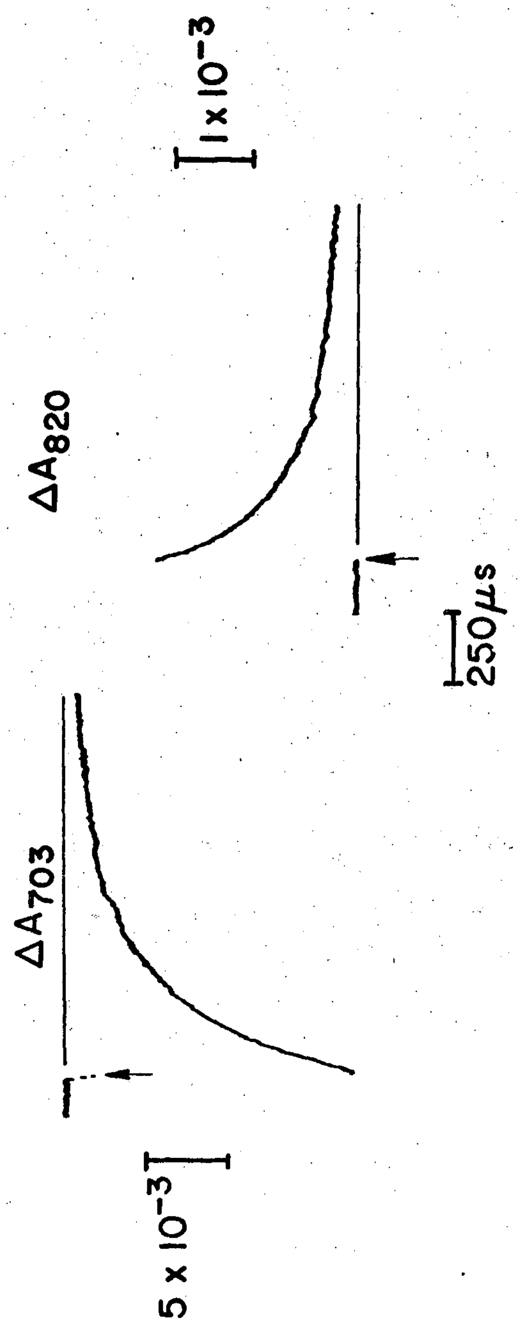
Third curve - two preillumination flashes; $t_{1/2} = 0.32$ ms (90%)

Bottom curve - three preillumination flashes; $t_{1/2} = 0.26$ ms (75%)

The response to only the last (dye laser) flash is shown. Each curve is the average of two signals.

Fig. 7. Difference spectrum for the flash-induced absorbances changes of broken chloroplasts from spinach. Anaerobic reaction mixture contains PMS (50 μM), sodium dithionite (2 mg ml^{-1}), tris buffer (0.2 M, pH 9.0) and chloroplasts sufficient to give $A_{678nm} = 0.9\ cm^{-1}$ or $1.7\ cm^{-1}$ (results from two experiments are shown). Background illumination was present. Amplitudes of an absorption component decaying with a half-time of about 3 μs are plotted; ordinate as in Fig. 2.

Fig. 8. Model showing the hypothetical arrangements of electron donor and acceptor components in Triton - solubilized Photosystem 1 particles.



XBL 7711-4771

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Fig. 1

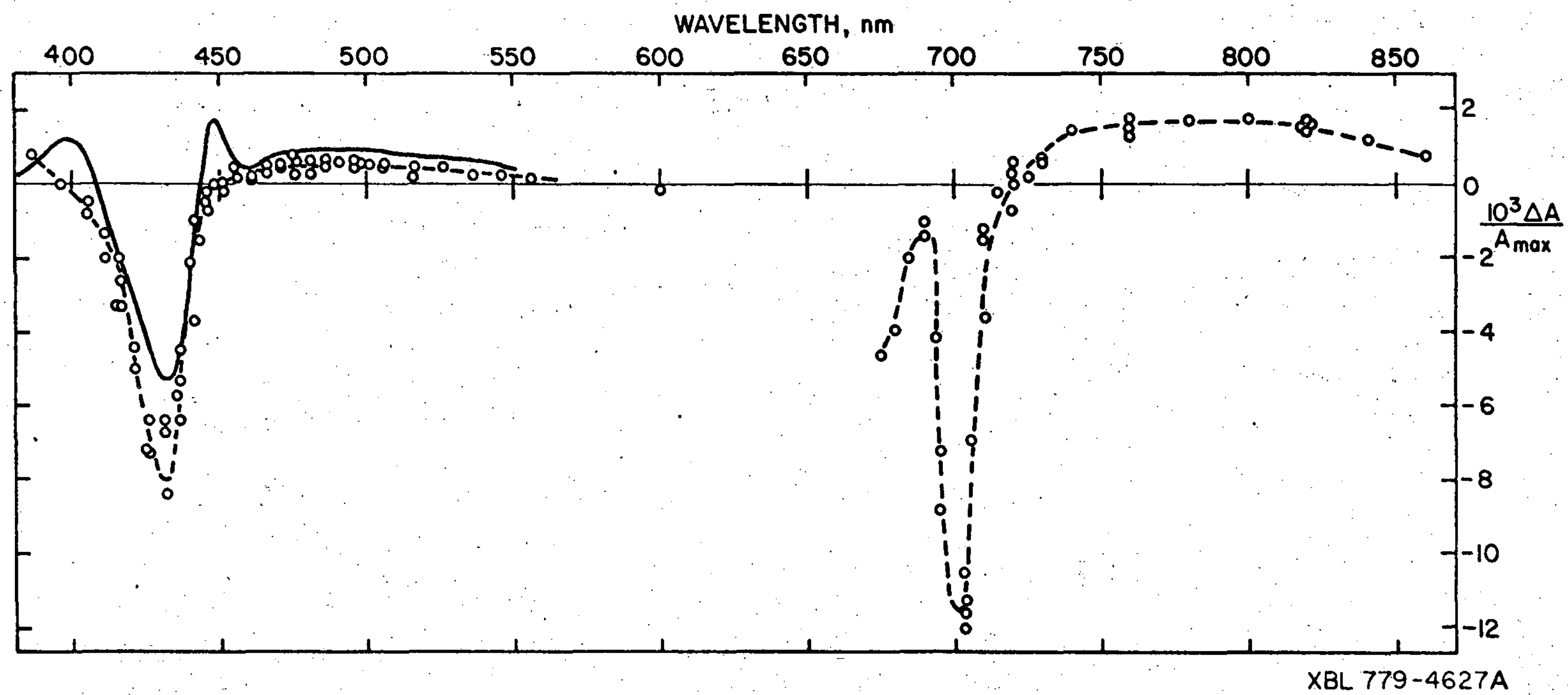

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Fig. 2

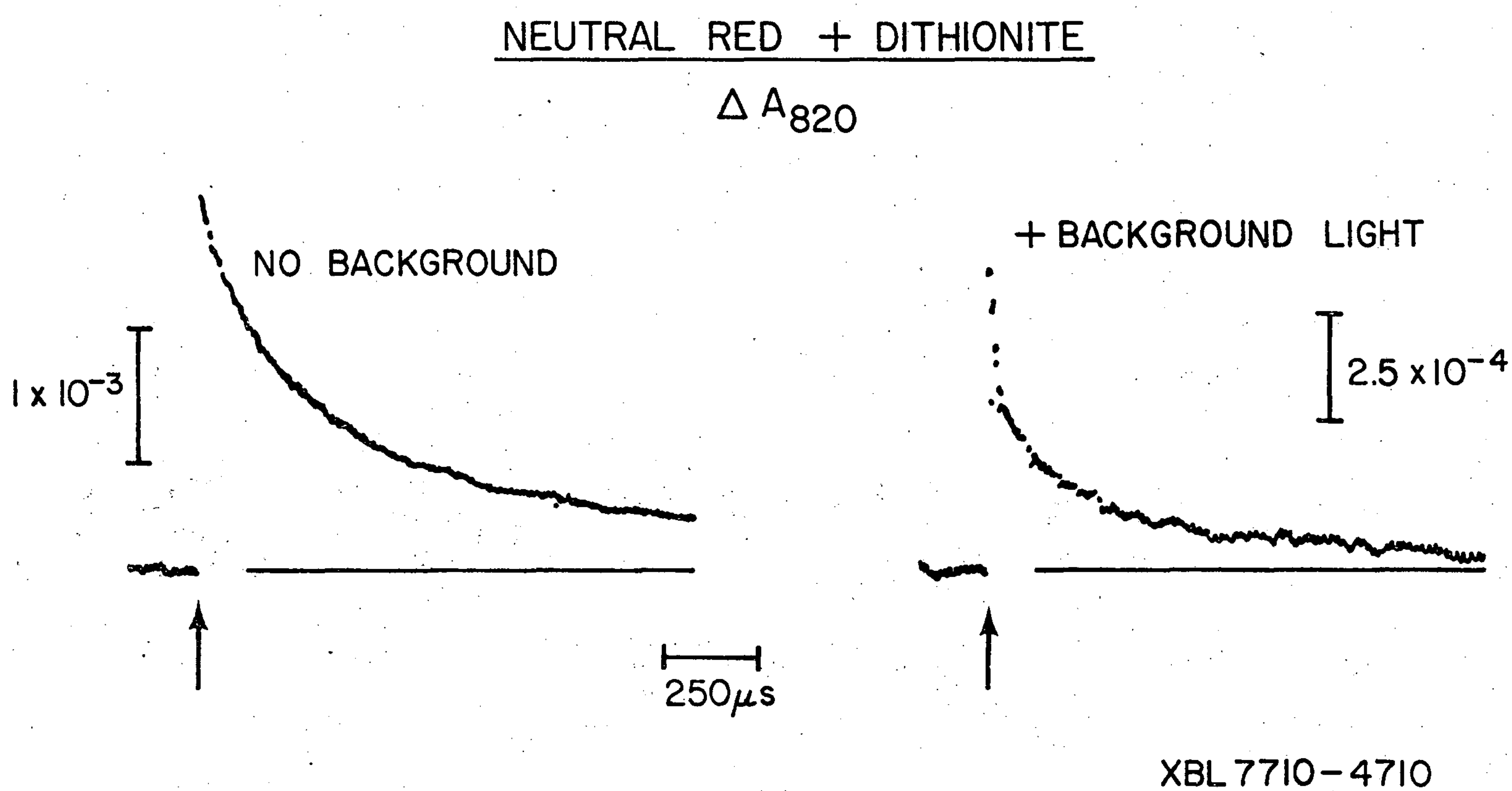

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Fig. 3

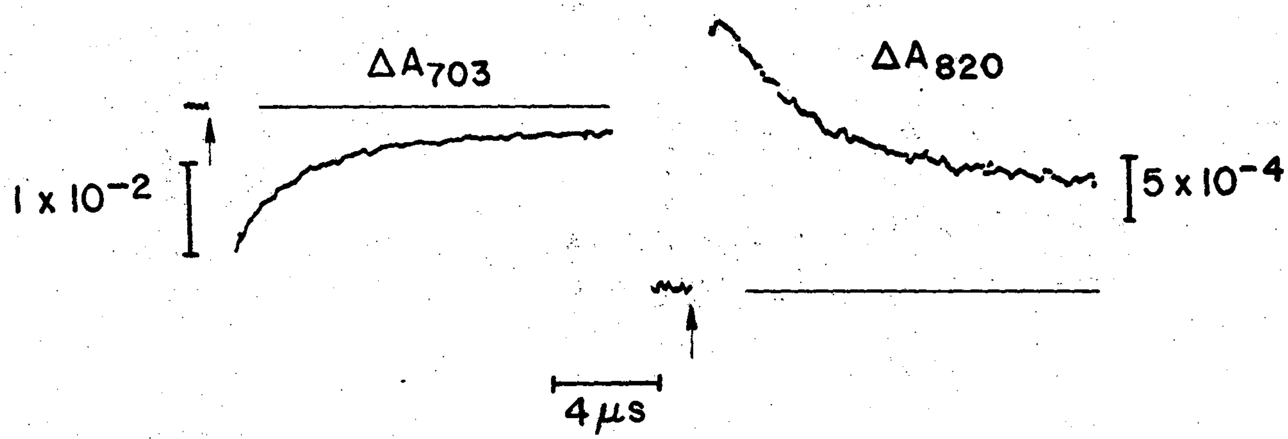

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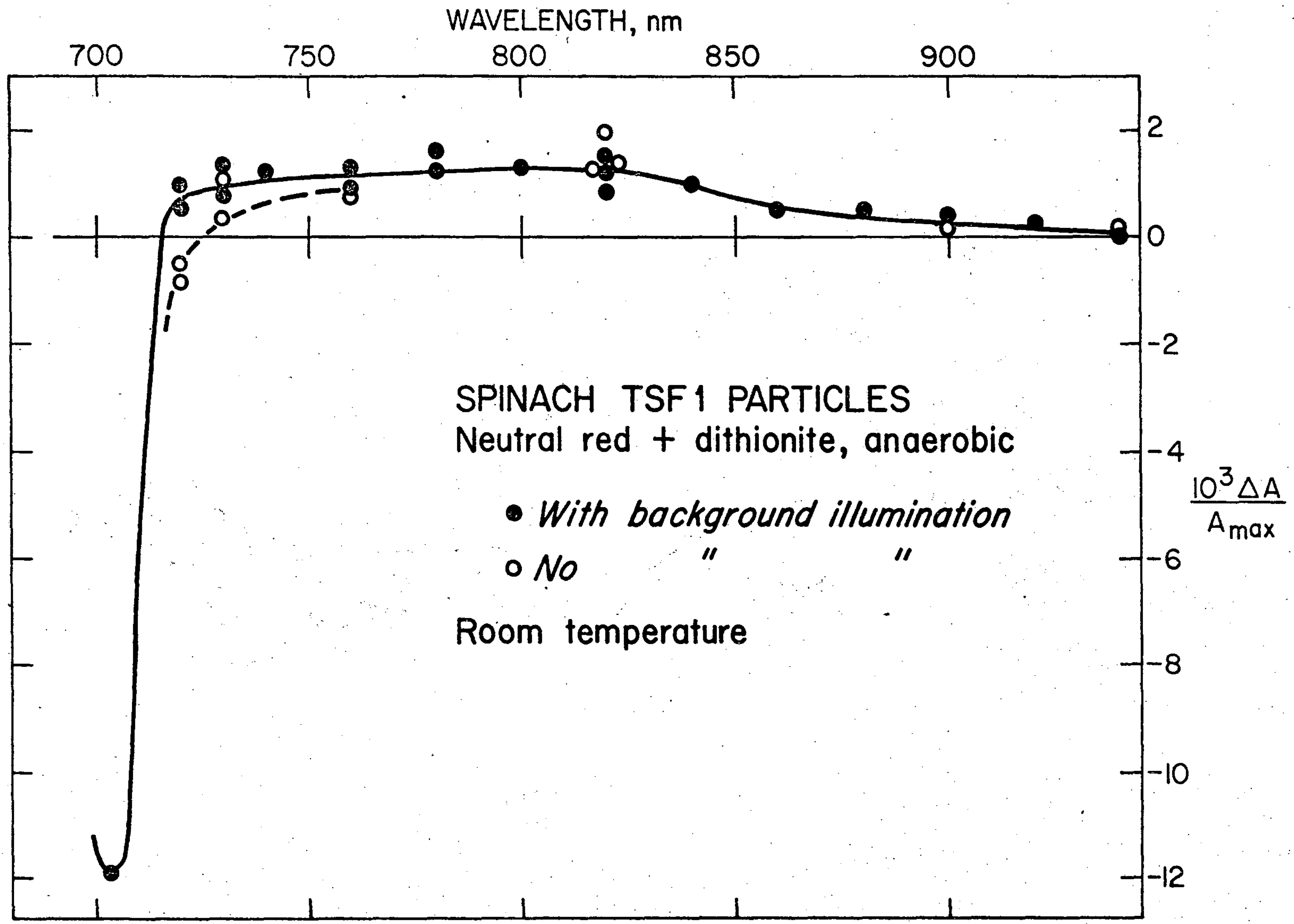
Fig. 4



XBL 779-4626

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Fig. 5

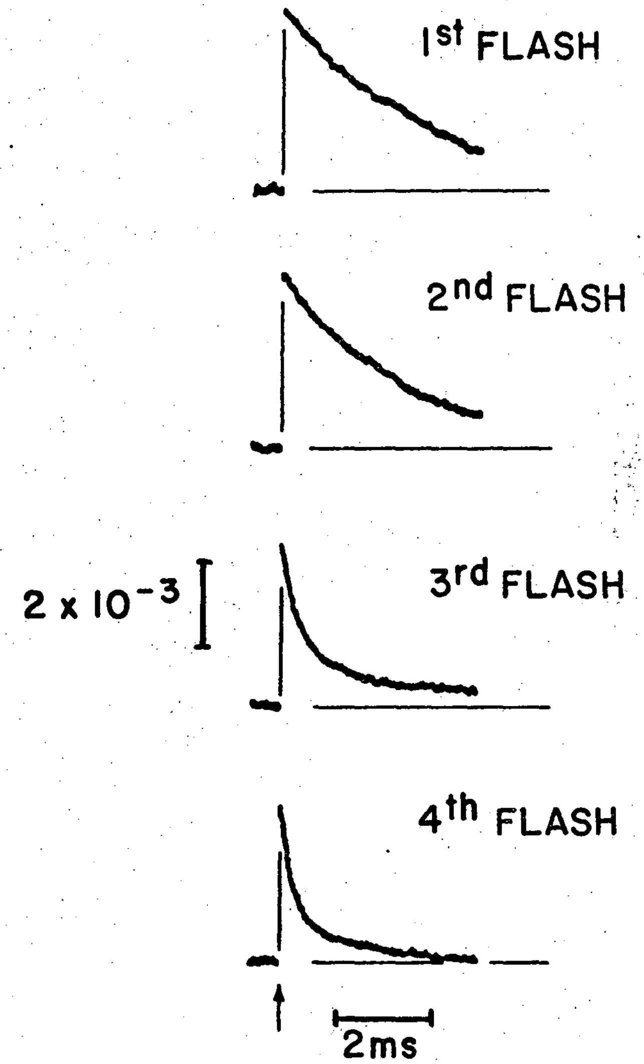

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Fig. 6

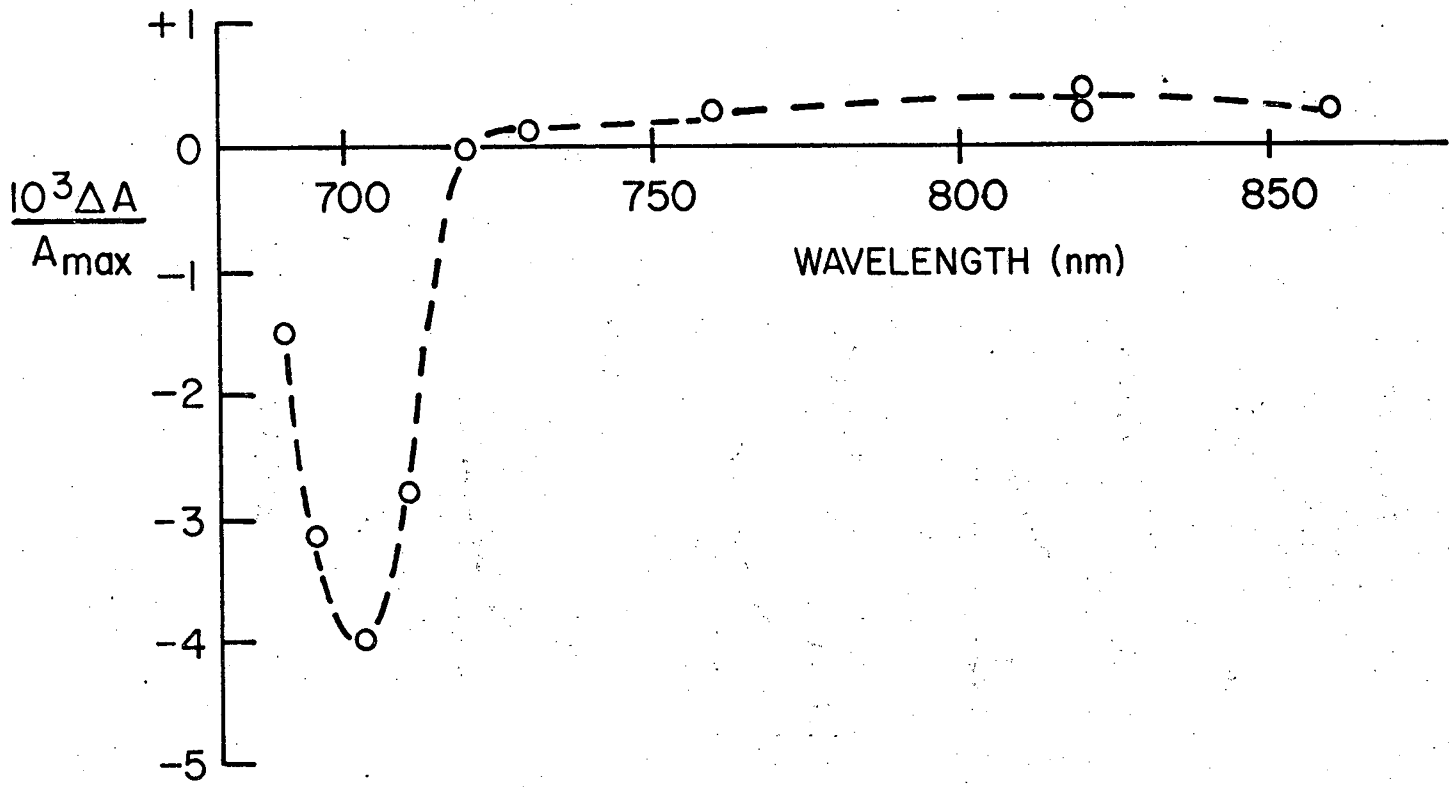

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Fig. 7

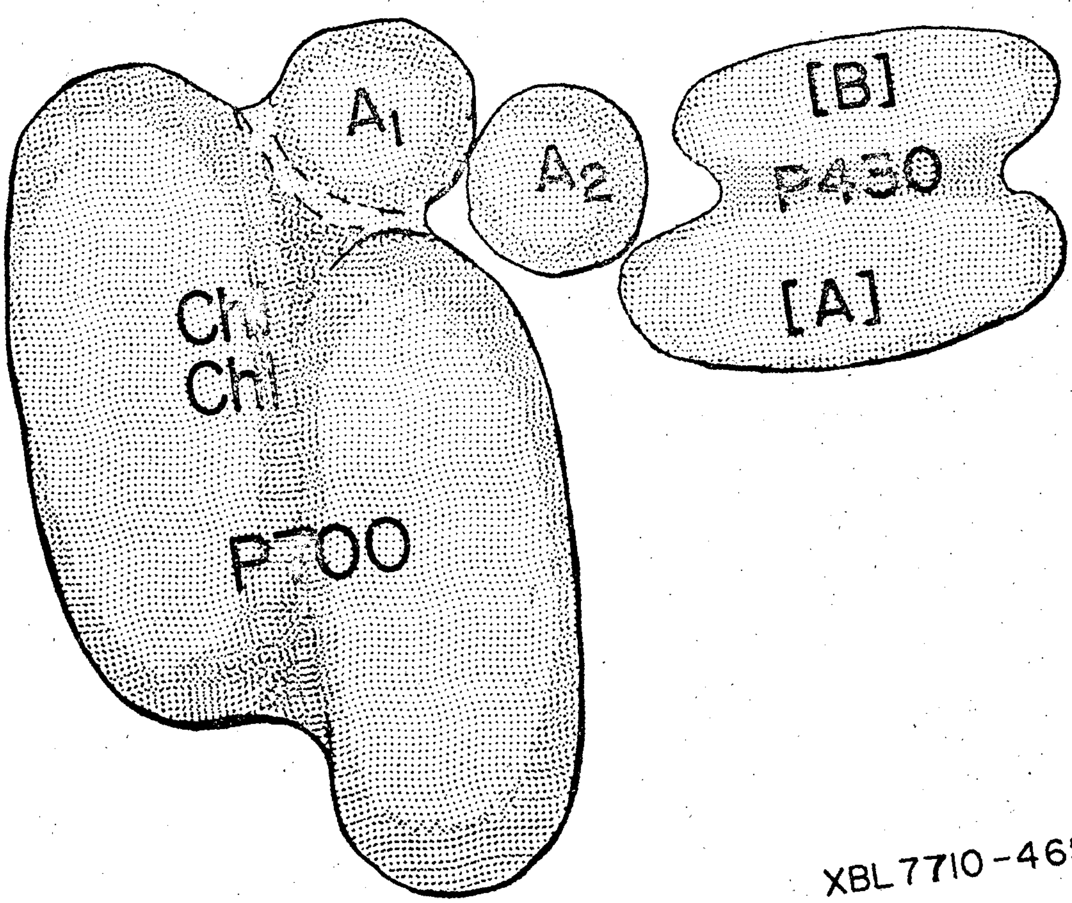

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Fig. 8

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