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A New Photosynthetic Pigment, "P430": Its Possible Role as the Primary Electron Acceptor of Photosystem I

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ABSTRACT **The technique of flash kinetic spectrophotometry was used to demonstrate a broad absorption band around 430 nm, which was kinetically different from P700, in several photosystem-I particles from spinach and blue-green algae. The component represented by this absorption band, designated as "P430", was bleached as fast as P700. Its recovery in the dark was accelerated by ferredoxin and by various artificial electron acceptors with redox potentials as low as −521 mV. The recovery kinetics have been demonstrated to be identical to those of a concomitant reduction of several of the artificial electron acceptors used. Tentatively, "P430" has been proposed as a possible primary electron acceptor of photosystem I.**

The identity of the primary electron acceptor in photosystem I of green-plant photosynthesis, a strong reductant that initiates the reduction of NADP, has been studied for many years, yet no definitive description about its nature has been reached. Arnon (1), among others (2, 3), has proposed ferredoxin as the primary acceptor. Very recently, Malkin and Bearden (4) have reported an ESR signal characteristic of reduced ferredoxin in spinach chloroplasts at 25°K after illumination at 77°K, and they suggested that some bound form of ferredoxin may serve as the primary acceptor. However, several other workers (5–8) were in favor of a more electronegative compound. Fuller and Nugent (9) have proposed pteridines as the primary acceptors for both green plants and bacteria. From a theoretical consideration, Kamen (10) has suggested that some form of chlorophyll could serve as the primary-acceptor molecule. Recently, Yocum and San Pietro (11) isolated a substance from spinach that stimulates NADP photoreduction. They suggested that this substance, "ferredoxin-reducing substance (FRS)", could be the primary acceptor. Subsequently, Regitz, Berzboron, and Trebst (12) reported a similar substance. The "cytochrome *c*-reducing substance (CRS)", isolated earlier by Fujita *et al.* (13), might also belong to the same category. In a model system, Wang (14) presented the possibility that a flavin compound could serve as the primary acceptor. Clayton and Straley (15) reported an absorption change around 450 nm in the reaction-center preparation of a mutant of *Rhodopseudomonas spheroides* that they proposed represented the primary acceptor. McElroy, Feher, and Mauzerall (16) detected an ESR signal at 1.4°K in the same reaction-center preparation that they ascribed to the reduced state of the primary acceptor.

In our previous study on P700 in the whole cells of a blue-green alga, we suggested that there was more than one spectral component in the Soret region that was bleached in less than 1 μsec by a ruby-laser flash (17). We have extended the work to cell-free systems in the presence of artificial electron donors and acceptors. In the course of this study, we found some anomaly in the re-reduction kinetics in the Soret region of P700 that had been photooxidized by a brief flash. We report here the observation of a spectral component that differs in many respects from either P700 or the cytochromes, and has characteristics attributable to the primary electron acceptor of photosystem I.

Abbreviation: TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine.

EXPERIMENTAL PROCEDURE

Sonicated fragments of *Plectonema boryanum* were prepared according to the method of Ogawa *et al.* (18). The method for the preparation of P700-enriched particles from acetone-extracted *Plectonema* fragments will be reported elsewhere. Two types of "high-P700 particles" from *Anabaena variabilis* were kindly supplied by Dr. T. Ogawa (19, 20). Photosystem-I particles from spinach chloroplasts were fractionated by digitonin treatment, according to the method of Anderson and Boardman (21). Chemicals were reagent grade and were used without further purification. Digitonin used in fractionation was recrystallized twice from ethanol. Ferredoxin was kindly provided by Dr. G. Palmer of the University of Michigan.

The technique of flash kinetic spectrophotometry was as described (17, 22). In most experiments, 20-μsec flashes from a xenon lamp were used as the excitation source. Red flashes were isolated by an interference filter that covered the spectral range 650–730 nm. Most light-induced absorption-change transients were averaged from 8 to 32 repetitive measurements in a Fabri-tek model-1062 signal averager to improve the signal-to-noise ratio. Detailed experimental conditions are described in the figure legends.

RESULTS AND DISCUSSION

The recovery phase of typical absorption changes at 430 nm induced by red flashes in P700-enriched particles from *Plectonema* is plotted, on a logarithmic scale, against time in Fig. 1. In the presence of both the electron donor (*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD), fully reduced by excess ascorbate) and the electron acceptor (methyl viologen), the time course of recovery in the dark was evidently biphasic; with 13 μM methyl viologen and 33 μM TMPD present, the rapid-decay phase had a half-time of less than 1 msec and the slow-decay phase 600 msec, as can be seen in Fig. 1*A*. The slow-decay phase had a simple exponential rate over a wide range of concentrations of the electron donor (TMPD), as shown in Fig. 1*B*.

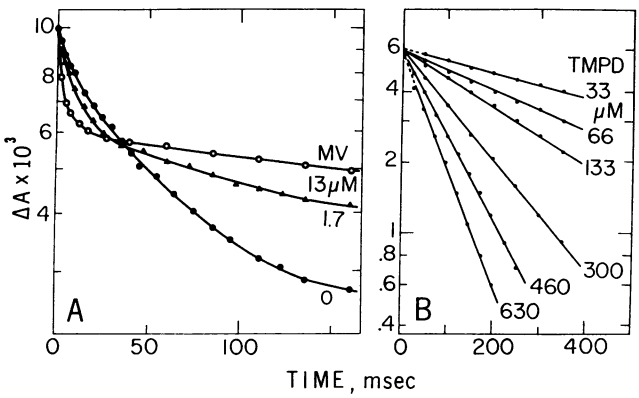

FIG. 1. Decay kinetics of absorption changes at 430 nm in P700-enriched particles from *Plectonema boryanum*. The reaction mixture, in 0.05 M Tris·HCl, pH 7.5, contained: (*A*) TMPD, 33 μM; sodium ascorbate, 0.67 mM; indicated concentrations of methyl viologen (MV). (*B*) methyl viologen, 13 μM; indicated concentrations of TMPD; sodium ascorbate, 0.67 mM. Temperature, 24°C. Chlorophyll concentration, 4 μg/ml. Light path, 1.0 cm. Each trace was the average of eight repetitive measurements.

The absorption changes of this slow-recovery phase at several representative wavelengths in the presence of methyl viologen and a given concentration of phenazine methosulfate, or of TMPD plus excess ascorbate, were plotted, on a logarithmic scale, against time in Fig. 2. These plots, when extrapolated to time zero, yielded the total magnitude of the slow-phase changes. The difference spectrum of the slow-phase change thus obtained is shown in Fig. 3. It should be noted that the slow-phase changes around 430 and 700 nm were kinetically identical, along with minor changes in other regions of the spectrum. This difference spectrum resembles closely those reported by Kok (23) and others (24, 25) for P700. From these results, we conclude that the slow-phase changes represent only the reduction in the dark of photooxidized P700 by the artificial electron-donor present. The exponential decay in

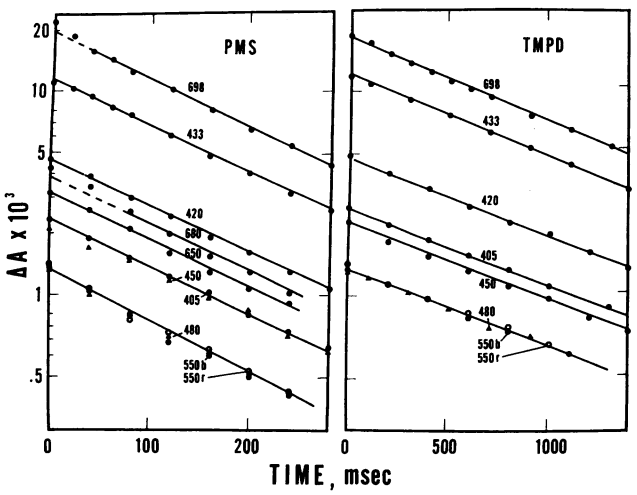

FIG. 2. Decay kinetics of absorption changes in P700-enriched particles from *Plectonema boryanum* at different wavelengths. The reaction mixture, in 0.05 M Tris·HCl, pH 7.5, contained: phenazine methosulfate (PMS), 1.6 μM (*left*); TMPD, 30 μM (*right*); sodium ascorbate, 0.6 mM; methyl viologen, 0.16 mM. Temperature, 24°C. Light path, 1.0 cm.

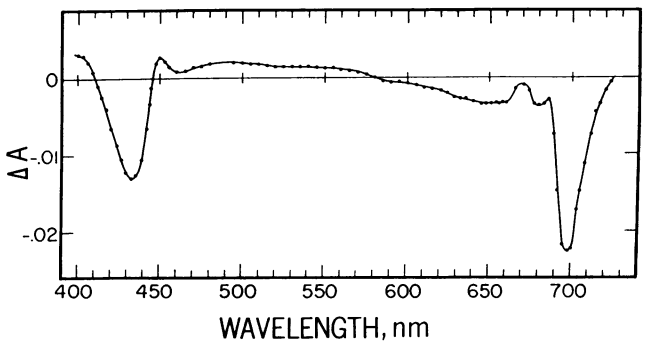

FIG. 3. Difference spectrum of P700 constructed from portions of the absorption changes showing exponential decay (see Figs. 1 and 2). The composition of the reaction mixture and the experimental conditions were the same as those described for Fig. 2 (*left*).

the presence of an excess amount of an electron donor indicates that the reaction is pseudo-first order, as has also been shown by Rumberg (26).

In the absence of methyl viologen, the slow-phase change was faster at certain concentrations of TMPD (see Fig. 1*A*). This may be explained as follows: in the absence of any artificial secondary electron acceptor, many of the electrons from the primary acceptor of photosystem I return to the photooxidized P700, as was suggested by Rumberg (26). This back reaction, which is visualized as the dark reduction of P700, can be faster than that dependent on an added artificial electron

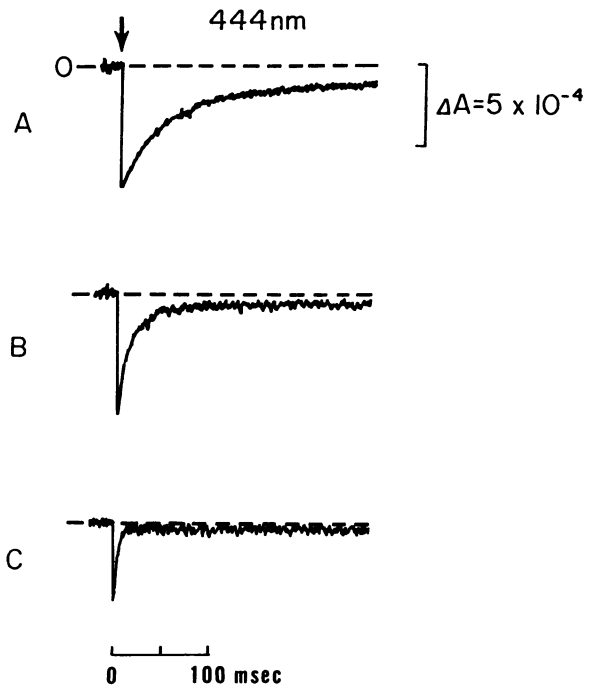

FIG. 4. Effect of methyl viologen on the kinetics of the rapid-decay component. The absorption changes were measured at 444 nm, a wavelength at which P700 contributes little light-induced absorption change. The reaction mixtures, in 0.05 M Tricine·NaOH, pH 8.0, contained: photosystem-I particles fractionated from spinach with digitonin (chlorophyll, 9.5 μg/ml): 2,6-dichlorophenolindophenol, 33 μM; ascorbic acid, 0.6 mM; and methyl viologen [none (*A*); 6.7 μM (*B*); and 67 μM (*C*)]. Each trace was the average of 32 repetitive measurements.

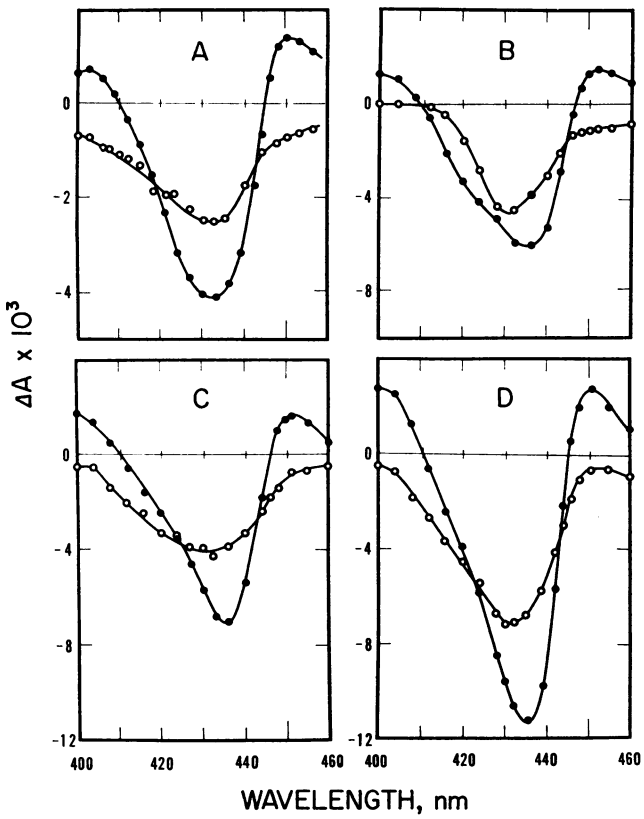

FIG. 5. Difference spectra of "P430" and P700. The composition of the reaction mixtures and experimental conditions were the same as in Fig. 1, except 0.05 M Tricine buffer, pH 8.0, was used instead of Tris. The samples were: (*A*) photosystem-I particles fractionated from spinach chloroplast with digitonin (21); (*B*) membrane fragments obtained by sonication of *Plectonema boryanum* cells; (*C*) high-P700 particles obtained from normally grown *Anabaena variabilis* by organic solvent extraction and Triton X-100 treatment (19); (*D*) high-P700 particles obtained from *Anabaena* cells grown in diphenylamine-supplemented medium (20). Chlorophyll concentrations were 15, 13, 18, and 11 μg/ml, respectively.

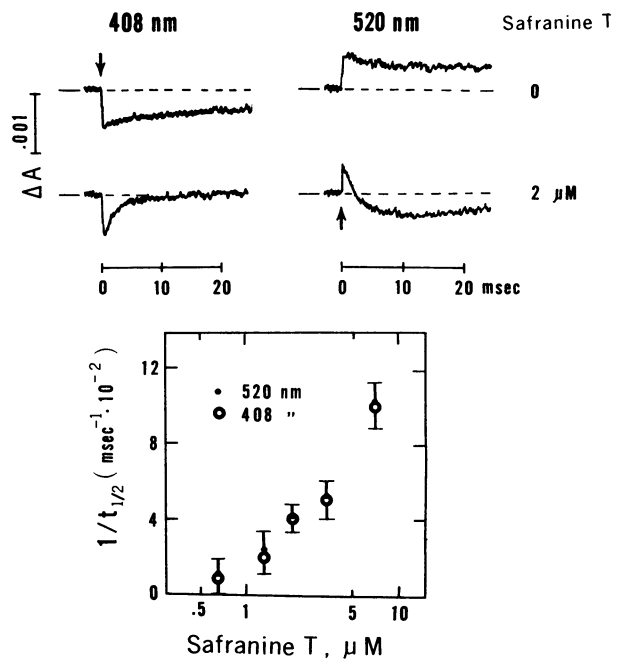

FIG. 6. Kinetics of absorption changes accompanying safranine T reduction and the concomitant "re-oxidation" of "P430". The absorption changes of "P430" in this case were measured at 408 nm, and that of safranine T reduction at 520 nm. The reaction mixture, in 0.05 M Tricine·NaOH, pH 8.0, contained: photosystem I particles fractionated from spinach chloroplasts with digitonin (chlorophyll, 13 μg/ml); TMPD, 33 μM; ascorbic acid, 0.67 mM; and the indicated concentrations of safranine T. Each trace was the average of 16 repetitive measurements.

donor. This is apparently the case in Fig. 1*A*, where a large part of the decay phase represents the dark reduction of P700 through the back flow of electrons from the reduced primary acceptor.

On the other hand, in the presence of methyl viologen, electrons from the primary acceptor are mostly channeled to this artificial secondary acceptor, and eventually to oxygen. As a result, the bulk of P700 that has been oxidized by a brief flash is re-reduced in the subsequent dark period only by the artificial electron donor present, and the reaction rate depends solely on the concentration of the donor, as shown in Fig. 1*B*.

By this reasoning, the rapid-decay phase was apparently due to some component other than P700. One remarkable feature of this reaction is that the rapid-decay phase became even more rapid in the presence of increasing amounts of methyl viologen. In Fig. 4, it is shown that the half-decay time of this component in the absence of methyl viologen was 35 msec in spinach photosystem-I particles, while it became 8 msec in the presence of 6.7 μM, and less than 1 msec at 67 μM methyl viologen. Note that we have chosen the wavelength 444 nm to examine the kinetics of this rapid-decay component, because, as shown in Figs. 3 and 5, P700 produced practically no light-induced absorption change at this wavelength; it is seen from Fig. 4 that absorption changes due to this rapid-decay component could be measured at 444 nm with little interference from P700.

The light-minus-dark difference spectra of the rapid-decay component in several particle preparations from blue-green algae and spinach are shown in Fig. 5, together with the difference spectra of P700. The P700 spectra (represented by *solid dots* in Fig. 5) were obtained as described above for Fig. 3. The difference spectra of the rapid-decay component (represented by *empty circles*) were obtained by subtracting the P700 spectra from the overall magnitudes of the initial rapid onset (in less than 1 msec) in the absence of methyl viologen. As seen from Fig. 5, the difference spectra of this rapid-decay component all show a broad band around 430 nm. We have, therefore, called this component "P430".

Several other artificial electron acceptors showed a similar effect on the back reaction and the acceleration of "P430" decay: 1,1′-trimethylene-2,2′-dipyridylium dibromide (E_0' value −521 mv, ref. 8); methyl viologen (−446 mv); benzyl viologen (−359 mv); safranine T (−290 mv); flavin mononucleotide (−219 mv); pyocyanine (−34 mv); methylene blue (+11 mv); 2,6-dichlorophenolindophenol (+217 mv); and *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (+260 mv). More interestingly, spinach ferredoxin, a known component of the reducing side of photosystem I, behaved similarly to the artificial electron acceptors in accelerating the decay of "P430".

We suggest that the absorption changes associated with "P430" represent the redox change of the primary electron acceptor of photosystem I of green plants and blue-green algae. Points in favor of this suggestion may be summarized as follows: (*a*) the onset time of the absorption change due to this component, less than 1 μsec, is fast and comparable to that of P700, as separately determined by excitation with a 20-nsec Q-switched ruby-laser flash; (*b*) the recovery rate of this component in the dark is accelerated by the presence of ferredoxin, the natural cofactor with the highest negative redox potential known, as well as by artificial electron acceptors covering a wide range of redox potentials; and (*c*) similar absorption changes have been observed in various photosystem-I particles. The observation of the acceleration of "P430" decay by ferredoxin indeed lends additional support to the suggestion that "P430" is the primary electron acceptor of photosystem I, leading to the eventual reduction of NADP.

Further supporting the above suggestion is the fact that the reduction of some artificial electron acceptors could be demonstrated to correspond kinetically to the decay or "re-reduction" of "P430". In the presence of safranine T as the secondary acceptor, as shown in Fig. 6, the absorption decreases at its maximum wavelength (520 nm), which represent the reduction of safranine T after a rapid positive-transient, correspond kinetically to the rapid decay at 408 nm. Note that we have chosen the wavelength 408 nm to measure the absorption change of "P430", because, as at 444 nm, P700 made practically no contribution to light-induced absorption change at this wavelength and, furthermore, the absorption change caused by the reduction of safranine T interfered much less at 408 nm than at 444 nm. The initial, rapid positive-transient at 520 nm was due to P700, which had a broad positive band around this spectral region, as can be seen in Fig. 3, where only the spectral component kinetically identical with P700 was shown. Note that under the experimental conditions used in Fig. 6, P700 decay was slow enough that it hardly interfered with the (rate of) absorption changes due to safranine T reduction at this wavelength. Positive changes in the 500-nm region have also been observed in previously reported P700 spectra (24, 27). Similar correspondence between dye-reduction kinetics and the decay kinetics of "P430" have also been obtained for tetramethylphenylenediamine (oxidized) and 2,6-dichlorophenolindophenol serving as secondary acceptors.

CONCLUDING REMARKS

Extensive measurements showed that the difference spectrum of "P430" had a broad band, ranging from 400 to 500 nm, with a maximum near 430 nm (details will be reported elsewhere). Preliminary measurements of light-induced absorption changes in the longer-wavelength region (500–800 nm), however, have not yielded detectable changes that were kinetically identical to those in the Soret region for "P430". Further investigations on the chemical nature of "P430", and more details about its role in the primary events in green-plant photosystem I, are in progress, with due consideration being given to those substances suggested in the current literature (1–4, 9–14).

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