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Minireview

P430: a retrospective, 1971–2001

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Abstract

The spectral species P430 and its spectral and kinetic properties are briefly reviewed. Currently available evidence shows P430 to be the optic-spectral representation of FeS-A/B, the electron acceptor(s) of Photosystem I (PS I).

Introduction

Following the discovery of the 'red drop' and the 'Emerson enhancement effect' phenomena in oxygen evolution (Emerson and Lewis 1943; Emerson et al. 1957), the concept of two photosystems in plant photosynthesis began to emerge (see J.M. Anderson, this issue). Soon the primary electron donors of Photosystems I and II were identified and named P700 and P680, respectively (see Ke 2001, pp. 479–504, 505–526). Toward the end of the 1960s, inquiry into the nature of the primary electron acceptor of Photosystem I also began. According to the prevailing notion at that time, the initial photochemical act in Photosystem I was to generate, through the absorption of a photon, a charge separation to produce a positively and a negatively charged species, or a 'charged pair.' However, the primary electron acceptor of Photosystem I was a rather elusive entity, subject mostly to speculation.

A physical model for a photosystem was first developed by E. Katz (1949) in terms of charge separation in a barrier photocell, and elaborated later by Calvin (1961). In this model, the photoexcited porphyrin donates an electron to an acceptor to produce a species with a high reducing level, which eventually then reduces CO_2, with the electron ultimately coming from water. The net result is the transfer of hydrogen from H_2O to CO_2.

Kamen (1961) described a possible mechanism for the coupling of a chemical reaction to the absorption of a photon by proposing the existence of a chlorophyll–heme adduct in the photosynthetic apparatus. Upon absorption of a photon, the excited state produces an electron transfer from the neighboring heme to the chlorophyll. The photooxidant and photoreductant then bring about the withdrawal of an electron from water and the reduction of the pyridine nucleotide.

The concept of chlorophyll serving as the primary electron acceptor was amended by Kamen (1963) using a model consisting of a chlorophyll dimer. The energy of a (red) photon removes an electron from one Chl of the [Chl·Chl] pair and places it on the other Chl to form [$Chl^+ \cdot Chl^-$]. Kamen's concept was prophetic and pertinent to all types of photosynthetic reaction centers to come under investigation later.

With the realization that ferredoxin, a component known to be present in all photosynthetic cells investigated and also a physiological reductant with the most negative redox potential known to be formed during photosynthesis, Arnon (1965) suggested that it was the 'crucial' electron acceptor in the primary photochemical act. Vernon and Ke (1966) further suggested the possible modes of ferredoxin reduction. In the meantime, however, three separate groups of investigators, Zweig and Avron (1965), Kok et al. (1965) and Black (1966), found that chloroplasts could reduce highly electronegative viologen dyes with E_0' values lying in the range of –500 to –700 mV, considerably more

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Figure 1. The Charles F. Kettering Research Laboratory in Yellow Springs, Ohio (mid-1960s).

Figure 2. The author (Bacon Ke) (1986).

Figure 3. The co-discoverer of P430 (Tetsuo Hiyama) (2001).

negative than the E_0' value of ferredoxin. These findings raised doubt that ferredoxin could be the primary reductant of Photosystem I.

Even though chlorophyll with a highly negative redox potential remained the most likely candidate to fulfill the requirement for the primary electron acceptor, other candidates continued to emerge. These included the naturally occurring, low-potential (∼–0.7 V), pteridine proposed by Fuller and Nugent (1969), a flavin-like compound suggested by Wang (1970), and 'ferredoxin reducing substance' (FRS) that was found to stimulate the photoreduction of

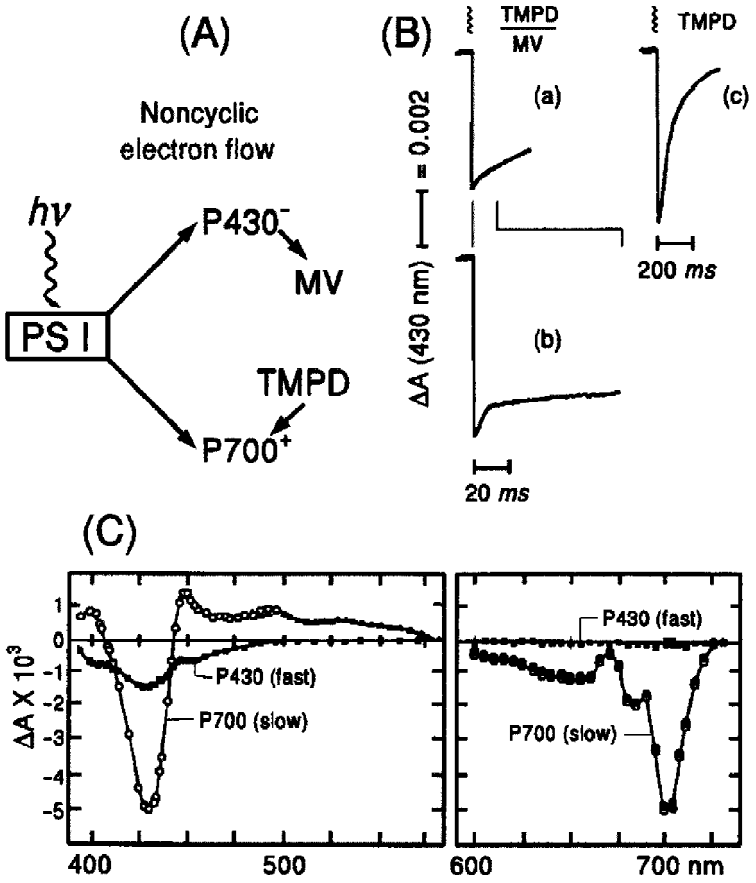

Figure 4. (A) The scheme of 'non-cyclic' electron flow in Photosystem I with reduced TMPD and methyl viologen serving as artificial electron donor and acceptor, respectively. (B) Flash-induced absorbance changes at 430 nm due to non-cyclic electron flow in digitonin-fractionated spinach Photosystem I particles (D144) measured at two different time resolutions [(a) and (b)] and in the presence of the reduced TMPD alone (c). (C) Difference spectra of P700 and P430 derived from kinetic plots made from the slow and rapid decaying signals obtained in the light-induced non-cyclic reaction as shown in (B, b). *Figure source*: Hiyama and Ke (1971a, b); Ke (1973).

$NADP^+$ and isolated by Yocum and San Pietro (1969) and by Regitz et al. (1970).

The turning point: discovery of P430

The turning point for the long search for the photosystem-I primary electron acceptor was the discovery of the membrane-bound iron–sulfur protein by Malkin and Bearden (1971) and the simultaneous discovery of the spectral species called 'P430' by Hiyama and Ke (1971a). These independent but apparently closely related investigations finally began to open up an active area of research of the Photosystem I reaction center. Figure 1 shows a photograph of the Charles F. Kettering laboratory (at Yellow Springs, Ohio), where this work was done, and Figures 2 and 3 show photographs of the authors of the 1971 paper (Bacon Ke and Tetsuo Hiyama), respectively.

The optic-spectral species P430 was discovered as a result of a missing kinetic component in the flash-induced transient absorbance change produced by a spinach Photosystem I subchloroplast fragment (D144), with ascorbate-reduced *N*, *N*, *N*′, *N*′-tetramethylphenylenediamine (TMPD) as an electron donor and methyl viologen (MV) as an electron acceptor (Figure 4A). At a relatively low time resolution, only a slow decay was observed (Figure 4B (a)). At an adequate time resolution, an additional small and rapid-decaying signal could also be detected (Figure 4B(b)). The slower ($t_{1/2}$ ~600 ms) decay and the small, rapid ($t_{1/2}$ ~ms) decays were, respectively, attributed to the dark reduction of $P700^+$ by reduced TMPD and the dark reoxidation of the pre-

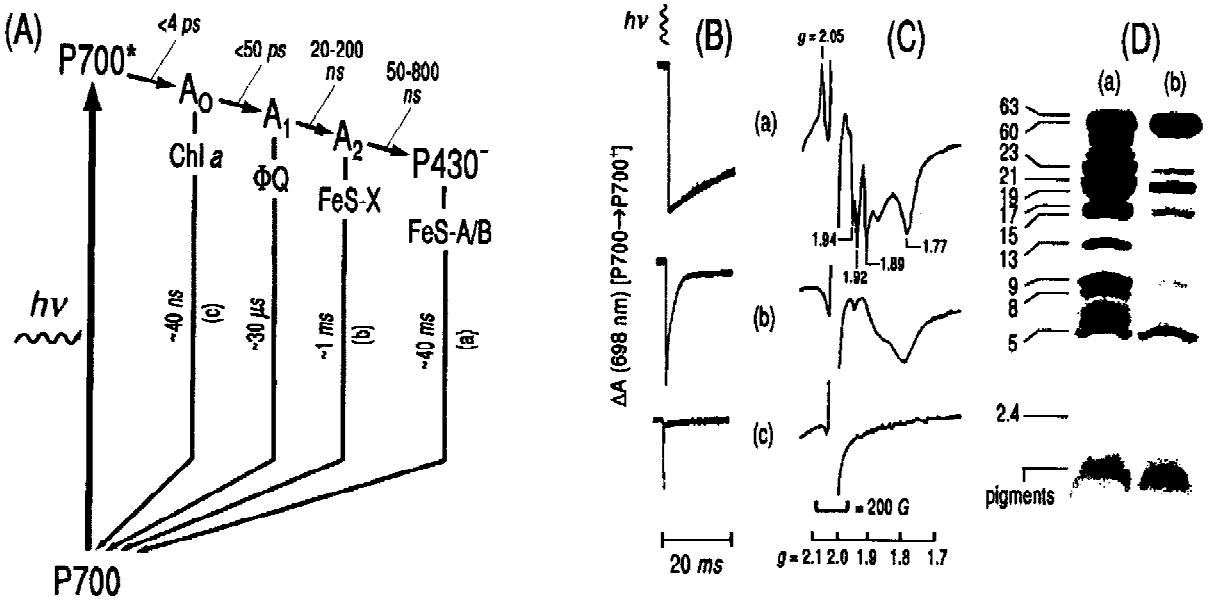

Figure 5. (A) Flash-induced absorbance changes due to P700 photooxidation and re-reduction of $P700^+$ with FeS-A/B^- (a), FeS-X^- (b) and Chl $a^-[A_0{}^-]$ (c), respectively. (B) EPR spectra of iron–sulfur centers measured at 10 K in native PS-I complex (a), PS-I complex incubated in ethylene glycol (EG) at 60 ° C for 5 min (b) and at 70 ° C for 5 min (c). (C) Polypeptide composition of the native PS-I complex (a) and that incubated in ethylene glycol at 60 °C for 5 min (b). Figures adapted from Hoshina and Itoh (1993).

sumed primary electron acceptor, $P430^-$, by methyl viologen in a 'non-cyclic' electron-transfer reaction. In the presence of reduced TMPD alone (Figure 4B (c)), $P700^+$ and the reduced primary electron acceptor ($P430^-$) are expected to undergo recombination in the dark ('charge recombination'); thus only a single kinetic component appears with a decay $t_{1/2}$ of approximately ~45 ms. At the measuring wavelength of 430 nm, the amplitude of the absorbance change of the single kinetic component in charge recombination is, as expected, equal to the sum of the slow and rapid decay components due to ($P700^+ \rightarrow P700$) and ($P430^- \rightarrow P430$) in a non-cyclic reaction.

The spectral profile of the primary donor P700 and the presumed Photosystem I primary acceptor may then be obtained by plotting the amplitudes of the slow and rapid decay phases for the D144 particles in the presence of reduced TMPD and MV, as shown in Figure 4C. The difference spectrum constructed from the slow-decaying changes represents the dark reduction of photo-oxidized $P700^+$ by the artificial electron donor TMPD. The difference spectrum constructed from the rapid-decaying changes shows mainly a broad band around 430 nm, plus some minor changes in the 400–500 nm region, and the component responsible for it was therefore called 'P430.' Little or no absorbance changes with rapid kinetics corresponding to those in the blue region could be found in the red region. Similar difference spectra were also obtained from Triton-fractionated particles from spinach and three kinds of P700-enriched particles derived from cyanobacteria (Hiyama and Ke 1971a).

There are five possible fates for the photo-oxidized $P700^+$ and the photoreduced $P430^-$ formed in the photochemical charge separation in Photosystem I under various reaction conditions. In addition to 'charge recombination' and 'non-cyclic electron flow,' the kinetic profiles of $P700^+$ and $P430^-$ in 'cyclic electron flow' and in the 'photo-accumulations' of either $P700^+$ or $P430^-$ were also demonstrated (Ke 1973; also see Ke 2001, pp 505–526).

Relationship of P430 to FeS-A/FeS-B centers

The 'spectral profile' of P430 naturally led Hiyama and Ke (1971a, b) and also Shuvalov, Klimov and Krasnovsky (1976) to suggest it to be related to an iron–sulfur protein on the basis of the resemblance of its difference spectrum to that of ferredoxin. Oh-Oka et al. (1990) later provided further support to the above suggestions by isolating the native FeS-A/FeS-B and measuring its absorption spectrum, and found it

to closely resemble that of P430 reported by Hiyama and Ke (1971a, b).

When $P430^-$ was initially found, it was thought to be the *direct* reaction partner of P700, i.e., the 'primary' electron acceptor of Photosystem I. As we now know, however, much faster electron transfers involving several 'earlier' acceptors [A_0, A_1 and FeS-X] actually precede the reduction of FeS-A/B, as illustrated in Figure 5A, but were not resolved because of the lack of adequate time resolution. Hoshina and Itoh (1993) presented an excellent summary of the chemical nature of the Photosystem I electron-acceptor chain together with their reaction kinetics, as presented in Figure 5. Their results strongly support the notion that P430 and FeS-A/B are different (optical and EPR) representations of the same electron carrier.

Figure 5A shows the kinetics of recombination of $P700^+$ with the reduced acceptors $[FeS\text{-}A/B]^-$ (a), $FeS\text{-}X^-$(b), and $A_0{}^-$(c) taking place in Photosystem I particles treated with ethylene glycol (EG) for 5 min at 25 °C (a), 60 °C (b), or 70 °C (c), respectively. It is clear that sample (a), in which FeS-A/B remained intact, as shown by the EPR spectrum in Figure 5B (a) and by the polypeptide composition of the sample shown in Figure 5C (a), the recombination time was ~30 ms. For the sample preincubated in EG at 60 °C, which retained the FeS-X center, as evidenced by the loss of the FeS-A/B polypeptide in the electrophoretogram in Figure 5C (b) and by the EPR spectrum in Figure 5B (b), $P700^+$ recombines with $FeS\text{-}X^-$ in ~1 ms. In the Photosystem I particle preincubated in EG at 70 °C, when FeS-A/B, FeS-X as well as the ΦQ (A_1) were removed, $P700^+$ recombines with Chl a^- (i. e. $A_0{}^-$) in ~40 ns (Figure 5A (c)).

The notion that P430 is the optical representation of FeS-A/B was corroborated also by the work of Parrett et al. (1989) in the preparation of the Photosystem I core complex [$P700{\cdot}A_0{\cdot}A_1{\cdot}FeS\text{-}X$]. The progress in achieving a pure core complex by incubating in a medium containing urea was monitored by the degree of removal of FeS-A/B. It was assumed that the amount of $P700^+$ decay with a $t_{1/2}$ of ~30 ms is a measure of the amount of FeS-A/B that still remained intact. Upon progressive removal of FeS-A/B, the amount of the 30-ms decay phase decreased and, when all FeS-A/B was removed (in ~30 min), only a ~1.2-ms decay phase, representing a faster recombination of $P700^+$ with $FeS\text{-}X^-$, remained.

In principle, a definitive identification of the optically detected P430 with FeS-A/B measured by EPR spectroscopy would best be made by spectro-kinetic

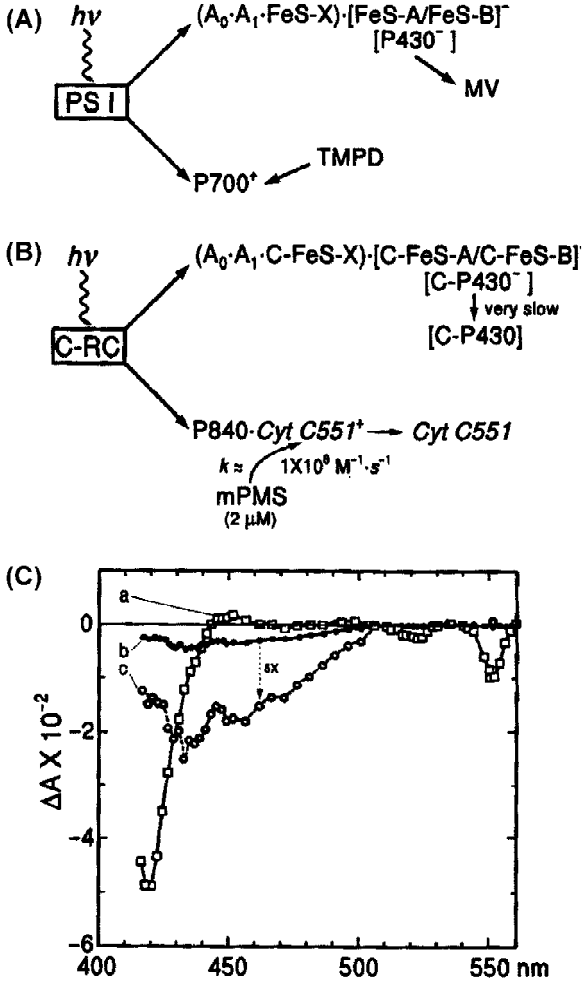

Figure 6. Schemes representing the components in the FeS-type reaction centers in Photosystem I (A) and the *Chlorobium* reaction center (*C*-RC) (B). (C) Difference spectra constructed from the recombination between *Cyt551*$^+$ and *C*-$P430^-$ (a); that for the accumulated *C*-$P430^-$ (b); spectrum (c) is (b) magnified five-fold. See text for descriptions. Figure (C) adapted from Kusumoto et al. (1995).

measurements. However, since the EPR signal of FeS-A/B is detectable only at ≤15 K when the decay times of $(FeS\text{-}A/B)^-$ are prohibitively long at this temperature, no meaningful, direct correlation is practicable. Low-temperature EPR spectroscopy has shown that the onset times of P700 photo-oxidation and FeS-A photoreduction are both rapid and essentially not resolvable under the rather limited time resolution used. Ke et al. (1974) carried out a series of 'freeze-quenching' experiments in an attempt to correlate P430 and FeS-A. The starting sample consisted of a dark-adapted Photosystem I particle cooled

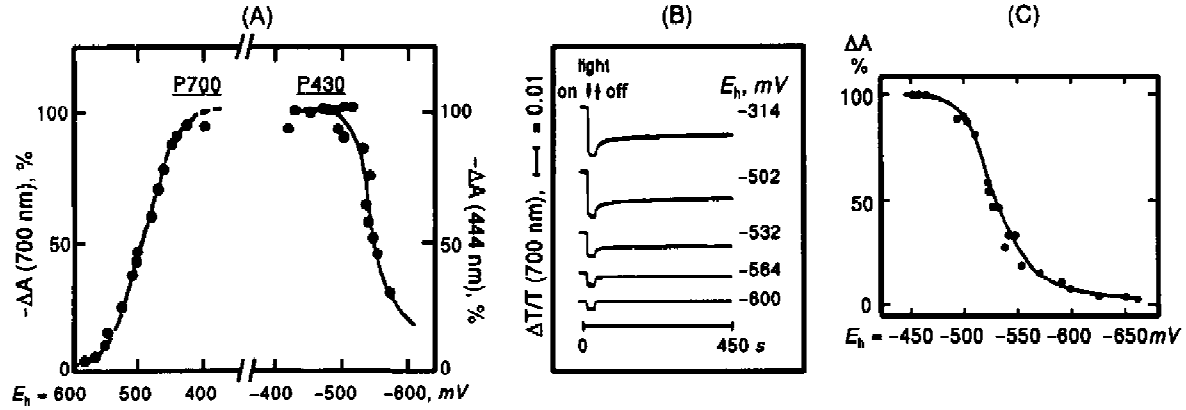

Figure 7. (A) Dependence of the amplitudes of light-induced absorbance changes due to P430 reduction (and also P700 oxidation); (B) Redox titration of PS-I particles (TSF-I) at pH 11 monitored by light-induced P700 absorbance changes at 86 K (shown for selected potentials); (C) Plot of the slow-decay component at 700 nm in (B) vs. the imposed redox potential. *Figure source*: (A) Shuvalov et al. (1976); (B, C) Ke et al. (1977).

to 13 K and illuminated to produce $P700^+$ and FeS-A^-. Such a sample was then rapidly brought to a higher temperature ranging from 75 to 225 K for time periods ranging from 25 s to 10 min, and then very rapidly quenched to 13 K for EPR analysis. In all cases, the extent of recovery, i.e. amounts of $P700^+$ re-reduced and FeS-A^- re-oxidized, was equivalent. The decay behavior estimated in this fashion agreed with that subsequently obtained from light-induced optical absorbance changes in Photosystem I at low temperatures (Ke et al. 1979).

A P430 analogue in green sulfur bacteria, C-P430

We now take note of the fact that green-sulfur bacteria and heliobacteria also have the FeS-type reaction centers, as shown in Figures 6A and B. The green-plant and green-bacterial systems differ, however, in that the secondary endogenous electron donor to P700, the copper protein plastocyanin, which is usually solubilized during the preparation of the reaction-center particle, the corresponding secondary donor to $P840^+$ in the green-sulfur bacterium *Chlorobium* (labeled '*C*-RC'), cytochrome *C551*, remains membrane-bound and photochemically active. Consequently, fast electron donation to $P840^+$from *C551* results in the eventual recombination of $(C\text{-FeS-A/B})^-$ or $C\text{-}430^-$ with $C551^+$ at a $t_{1/2}$ ~400 ms, and a difference spectrum for $[C551^+–C551]+[C\text{-}P430^- – C\text{-}P430]$ (Figure 6C(a)), where *C*-P430 and *C551* cannot be spectroscopically or kinetically differentiated. Kusumoto et al. (1995), using the reaction-center complex isolated from the green-sulfur bacterium *Ch. tepidum*, devised another reaction pathway for accumulating $C\text{-}P430^-$. They used mPMS (1-methoxy-5-methylphenazonium methylsulfate), an efficient electron mediator, to intercept the oxidizing equivalent from $C551^+$, whereby re-oxidation of $C\text{-}P430^-$ by $C551^+$ was prevented, and a difference spectrum of the accumulated $C\text{-}P430^-$(i.e. $[C\text{-}P430^- – C\text{-}P430]$) resulted (Figure 6C(b) and (c)).

Comparison of P430 and FeS-A/B based on redox potentiometry

P430 and FeS-A/B may also be correlated on the basis of their redox potentials. The E_m values of FeS-A/B determined directly by redox potentiometry and monitored by EPR spectroscopy (Ke et al. 1973; Evans et al. 1974) have been used as the basis for comparison. At first, only the approximate E_m value of P430 at room temperature was estimated to be between –500 and –600 mV by Hiyama and Ke (1971b) on the basis of its ability to reduce certain exogenous electron carriers, including the low-potential viologen dyes.

Shuvalov et al. (1976) later determined the redox potential of P430 directly by monitoring the amplitude of its light-induced absorbance changes at 444 nm (near an isosbestic point of P700) at room temperature as a function of the redox potential imposed on a Photosystem I particle (DT175). As shown by the titration curve in Figure 7A, the absorbance-change amplitude due to P430 remained unchanged when the potential was decreased to –500 mV, began to decrease below

–500 mV, with just about 50% of the signal remaining at –550 mV, agreeing with the E_m value determined directly for FeS-A by EPR-potentiometry.

Subsequently, Ke et al. (1977), in an attempt to correlate P430 with the membrane-bound FeS-A/B, measured the light-induced changes in both the optical and EPR (not shown) spectra of P700 at 86 K as a function of the imposed potential. As shown in Figure 7B, the biphasic decay phase consists of ∼20% rapid-decaying phase, which stayed constant over a wide range of potentials, plus a virtually irreversible phase whose amplitude is dependent on the redox potential imposed on the sample. At the most negative potential of –600 mV, the residual signal was almost exclusively of the rapidly decaying type. The titration curve constructed from the amplitude of the slowly decaying signal vs. potential is shown in Figure 7C. The midpoint potential for the species being chemically reduced and thus unavailable to participate in the photochemical charge separation was estimated to be ∼ –530 mV, in agreement with the value obtained previously by direct EPR titration of FeS-A (Ke et al. 1973).

Concluding remark

In conclusion, it can be said that there is ample evidence to support the notion that P430 is the optical representation of the iron–sulfur protein(s) FeS-A/B. It should also be noted that, at the moment, the precise reaction pathway of FeS-A and FeS-B themselves in the electron-transfer chain of Photosystem I is under intensive investigation. A more detailed correlation between P430 and FeS-A and FeS-B also remains open (see Ke 2001, pp 479–504).

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