Defining the Far-red Limit of Photosystem I

**THE PRIMARY CHARGE SEPARATION IS FUNCTIONAL TO 840 nm**

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Background: It is generally accepted that Photosystem I is functional under illumination up to 760 nm.

Results: We show that Photosystem I photochemistry is induced by far-red photons up to 840 nm.

Conclusion: The limit of Photosystem I is 80 nm more red-shifted than previously thought.

Significance: Charge transfer bands associated with P_{700} could be responsible for this effect.

The far-red limit of photosystem I (PS I) photochemistry was studied by EPR spectroscopy using laser flashes between 730 and 850 nm. In manganese-depleted spinach thylakoid membranes, the primary donor in PS I, P_{700}, was oxidized simultaneously with tyrosine Z, the secondary donor in PS II. It was found that at 295 K PS I photochemistry, observed as P_{700} \textit{formation}, was functional up to 840 nm. This is 30 nm further to the red region than was reported for PS II photochemistry (Thapper, A., Mamedov, F., Mokvist, F., Hammarström, L., and Styring, S. (2009) \textit{Plant Cell} 21, 2391–2401). The same far-red limit for the P_{700} \textit{formation} was observed in a PS I reaction center core preparation from \textit{Nostoc punctiforme}. The reduction of the acceptor side of PS I, observed as reduction of the iron-sulfur centers F_A and F_B by low temperature EPR measurements, was also functional at 15 K with light up to >830 nm. Taken together, these results, obtained from both plants and cyanobacteria, most likely rule out involvement of the red-absorbing antenna chlorophylls in this reaction. Instead we propose the existence of weak charge transfer bands absorbing in the far-red region in the ensemble of excitonically coupled chlorophyll \textit{a} molecules around P_{700} similar to what has been found in the reaction center of PS II. These charge transfer bands could be responsible for the far-red light absorption leading to PS I photochemistry at wavelengths up to 840 nm.

Photosystem I (PS I) is a multisubunit protein complex located in the thylakoid membrane of plants, algae, and cyanobacteria (1). The primary role of PS I is the light-driven oxidation of the luminal electron carrier protein plastocyanin and the reduction of the cytosolic (stromal) electron mediator protein ferredoxin (2, 3). The reaction center protein core of PS I consists of two membrane-spanning subunits, PsA and PsB, and one peripheral subunit, PsC, located on the stromal side of the membrane (2, 4–6). The PsA and PsB subunits together coordinate ~100 Chl \textit{a} and ~20 β-carotene molecules (2, 7, 8). These Chls compose the inner PS I antenna that harvest light and transfer the light energy to the reaction center through excitonic energy transfer, whereas the β-carotenes, in addition to light harvesting, also have a photoprotective role during high light conditions (8–11). The redox cofactors of the electron transfer chain of PS I are also bound by the PsA and PsB subunits: six Chls and two phylloquinone molecules coordinated in two symmetrical branches denoted A and B (2, 4–6, 8). In addition, PS I has three iron-sulfur centers denoted F_X, F_A, and F_B; the last two are bound to the PsA subunit on the stromal side of the membrane (2, 4, 8–10).

The crystal structure of the PS I reaction center in cyanobacteria is solved to 2.5 Å and in plants to 4.4-Å resolution (12, 13). The PS I complex from cyanobacteria exists in a trimeric form where one monomeric reaction center unit contains 12 different subunits, nine transmembrane proteins (together with the reaction center core proteins PsAa and PsBb), and three peripheral stromal proteins (including PsaC) (2, 4, 6, 7). The PS I reaction center from higher plants does not form trimers and exists in the monomeric form only (4–6). The monomeric reaction center complex in higher plants contains four additional protein subunits with functions that are not clear at the moment (2, 4–6). The different macromolecular composition in part reflects the very different outer antenna organization between plants and cyanobacteria.

Cyanobacteria lack the membrane-bound outer antenna completely. Instead they have a soluble antenna system, the phycobilisome, which contains protein-bound phycocyanin and phycoerythrin, depending on species and growth conditions, instead of Chls and carotenoids. The phycobilisomes are water-soluble and quite loosely attached to the stromal side of the thylakoid membrane (14–16). In contrast to cyanobacteria, the outer antenna in higher plants have up to six membrane-bound light-harvesting proteins denoted lhca1–6 (LHC I) in close association with the reaction center complex (4–6). Both phycobilisomes and LHC I harvest and transfer light energy via excitation energy transfer to the reaction center of PS I.

When excitation energy has reached the reaction center of PS I, the charge separation takes place between the excitonically...
coupled primary donor Chls \( P_A \) and \( P_B \) (denoted \( P_{700} \) after its absorption maximum) and Chl \( A_0 \) within 1–3 ps (8–10). The electron is further transferred to the phylloquinone A within 30 ps and then in the nanosecond time scale to the iron-sulfur centers \( F_x \), \( F_A \), and \( F_B \) (8–10). The final step in the PS I electron transfer reactions takes place with reduction of ferredoxin by \( F_B^- \) in the microsecond time range (8–10). The electron transfer components in the reaction center of PS I are coordinated to two almost identical branches, A and B (Scheme 1). After excitation of \( P_{700} \), the electron can potentially be transferred through either one of the branches, but the B branch is slower and less effective (9, 10, 17).

The oxidized primary donor, \( P_{700}^+ \), is reduced by electron transfer from the soluble luminal protein plastocyanin on the millisecond time scale under physiological conditions in plants (18). In the absence of reduced plastocyanin, electron donation to \( P_{700}^+ \) is absent, and a long lived radical from \( P_{700}^+ \) is detectable by EPR spectroscopy (19). This EPR radical signal, which was first described in Commoner et al. (20) and further characterized in Kok and Beinert (19), has a width of 7 G. This is 3–4 G more narrow than the EPR spectrum from a monomeric cationic Chl radical that, however, has the same \( g \)-value of 2.0026 (19).

In their classical work, Emerson and Lewis (21) demonstrated that the quantum yield of photosynthesis dropped drastically when using excitation light above 700 nm. This was later recognized as the absorption maximum of PS I. This phenomenon is now referred to as the Emerson effect or red drop of photosynthesis (1, 21, 22). However, light absorption above 700 nm has also been observed. LHC I is able to absorb light above 700 nm via two interacting Chls known as the “red Chls” (long wavelength Chls) (23, 24). The role of these red Chls is currently under discussion (25–29). The inner antenna of PS I in cyanobacteria also harnesses some red-shifted Chls (30). These are thought to be located at the interface between the monomeric units in the trimeric reaction centers (30, 31). The cyanobacterium *Spirulina platensis* can upon PS I trimerization form red Chls that absorb around 715 nm at 295 K (30). In both plants and cyanobacteria, the red-shifted Chls are considered to consist of a pair of close lying molecules that are excitonically coupled. This gives rise to Chls with a charge transfer-like excited state that can absorb a photon of a lower energy than the monomeric pigment exited state, thereby giving rise to the far-red light absorption (32). Similarly coupled Chls also exist in reaction center pigment assembly of PS I, PS II, and the bacterial reaction center (33–37). They have been reasonably well studied in the PS II reaction center, but less is known about such states in PS I. It is worth mentioning that it has been shown that the PS II reaction center can undergo charge separation with light up to 800 nm at both room (295 K) and very low (5 K) temperatures (38–41).

Recently, we reported that light-induced photochemistry can be observed in PS I at room temperature up to 840 nm (42). In the present work, we extend this initial study and follow the formation of the oxidized primary donor in PS I, \( P_{700}^+ \), and reduction of the FeS terminal acceptors by EPR spectroscopy also at low temperature. We induced these effects in both cyanobacteria and higher plants with different antenna compositions and therefore isolated the phenomena to the reaction center complex of PS I. Based on comparisons with PS II, a possible mechanism involving charge transfer states in the PS I reaction center is suggested to be involved in this far-red photochemistry in PS I.

### EXPERIMENTAL PROCEDURES

**Thylakoid Membrane Preparation and Manganese Depletion**—Spinach (*Spinacia oleracea*) was grown hydroponically as described earlier (43). The thylakoid membranes were isolated as described (44) and resuspended in a buffer containing 15 mM MES-NaOH, pH 7.0, 300 mM sucrose, and 15 mM NaCl for storage at −80 °C at a Chl concentration of 4 mg/ml.

To remove the CaMn4 cluster and extrinsic subunits in PS II, the thylakoid membranes were incubated in 1.0 M Tris–HCl, pH 9.0 at Chl concentration of 1 mg/ml under room light at 4 °C under constant stirring for 30 min as described (45). After washing, the thylakoids were resuspended in a buffer containing 25 mM MES, pH 6.1, 400 mM sucrose, 15 mM NaCl, and 3 mM MgCl₂. The same buffer was used in the measurements. The obtained material was diluted to a Chl concentration of ~2 mg/ml and kept at −80 °C.

**Photosystem I Reaction Center Isolation**—The cyanobacterium *Nostoc punctiforme* strain ATCC 29133-S (also known as UCD 153 (46, 47)) was grown in BG11 medium (48) at 25 °C under white light (30 μmol m⁻² s⁻¹) in stirred liquid cultures. Prior to thylakoid isolation, batch cultures in liquid medium were scaled up to 1.5 liters, grown under continuous stirring, and sparged with air for 7 days. Cells from 1.5-liter liquid cultures were harvested, and thylakoid membranes were isolated from the cells essentially as described (49). The preparation used here was made from PS I containing a decahistidine tag in the C terminus of the PsbP protein.4 The His-tagged PS I reaction center was prepared from the thylakoid membranes using immobilized metal ion affinity chromatography on a chelating resin column. The His-tagged PS I reaction centers were eluted by washing with imidazole buffer (15 mM). The buffer was exchanged to 20 mM Hepes–NaOH, pH 7.5, 10 mM MgCl₂, 10 mM CaCl₂, and 10% glycerol (49), and the His-tagged PS I reac-

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4. A. Magnuson and P. Oliviera, personal communication.
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RESULTS

Wavelength-dependent Radical Formation at 295 K in Manganese-depleted Thylakoid Membranes—Manganese-depleted thylakoid membranes from spinach were exposed to monochromatic laser flashes ranging from 532 to 840 nm at 295 K. EPR spectra were recorded prior to illumination and during continuous flashing. Typical results are presented in Fig. 1. In the dark, the EPR spectrum is dominated by the long lived radical signal from YD in PS II (Fig. 1A, spectra i). In membranes that are dark-adapted for a few minutes, YD amounts to one radical per PS II reaction center and can be used as an internal standard for quantification of other radical species formed in the same samples (43). In our experiments, this corresponds to the dark spectrum a in Fig. 1A. However, in manganese-depleted thylakoid membranes, YD decays slowly in the dark during the course of the experiment, which took several hours to conclude. This resulted in different amplitudes of YD in the “dark spectra” in the rest of Fig. 1A. Illuminating the membranes at 295 K can give rise to two additional EPR signals dependent on excitation wavelength (Fig. 1A, spectra ii). One originates from YZ in PS II that decays slowly enough to be detected by standard EPR spectroscopy in the absence of the CaMn4 cluster (50–52). The spectrum from YZ is identical in shape to the spectrum from YD, and is directly observable in the spectra in Fig. 1, A and B, as a shoulder at 3445 G where there is no overlap from other radical species. The second radical that is formed in manganese-depleted thylakoid membranes is P700+ from the oxidized primary donor in PS I. This is observable as a narrow radical signal in the middle of the EPR spectra in Fig. 1, A and B (marked with a bar).

Fig. 1A displays the radical EPR spectra recorded in the dark (i) and during illumination (ii) at 532 (spectra a), 730 (spectra b), 780 (spectra c), and 820 nm (spectra d). Before illumination, the EPR spectrum is dominated by the dark stable YD (see above). In the spectrum recorded during illumination at 532 nm (spectrum a, ii), two additional species were observed: the light-induced signal from YZ indicated with an asterisk at 3445 G and the narrow radical signal from P700+ indicated with a bar.
at 3445 G that is overlaid on the EPR spectra from both YZ* and YZ'. In the spectra recorded after illumination with light in the far-red region, YZ* could only be observed in the spectrum recorded at 730 nm (spectrum b, ii), whereas it was absent from the spectra recorded at 780 and 820 nm (spectra c and d, ii). In contrast, the narrow radical from P700* was observable in the light-induced spectra at all wavelengths used in Fig. 1A.

To obtain clean and quantifiable spectra of YZ* and P700*, the EPR spectra from Fig. 1A were deconvoluted. To quantify the EPR signal from YZ*, we subtracted the EPR spectrum recorded in the dark from the spectrum recorded during flash illumination (Fig. 1B). Thereby the dark stable signal from YD* was removed. The shoulder observable at 3445 G now represents YZ* only (Fig. 1B, marked with *), and there is, at this field position, no overlap with the EPR spectrum from P700*. It can thus be used to quantify YZ* without further deconvolution. To obtain quantifiable EPR spectra of P700*, the spectra were further deconvoluted by subtraction of a weighted fraction of YZ* from the spectra in Fig. 1B. The resulting spectra, reflecting P700* alone, are shown in Fig. 1C.

Quantification of the Far-red Light-induced Radical Signals—Fig. 1C (spectrum ox) shows the EPR spectrum obtained by quantitative oxidation of P700 by ferricyanide. Fig. 1C also shows P700* formed by illumination at 532 (spectrum a), 730 (spectrum b), 780 (spectrum c), and 820 nm (spectrum d). The line width (7 G) and g-value of the chemically induced P700* (spectrum ox) and the light-induced narrow radical signals (spectrum a-d) are identical. This is marked with bars in Fig. 1C and clearly demonstrates that the narrow radical species induced at all wavelengths originate from P700*.

The fraction of light-induced P700* radicals per PS I reaction center at different wavelengths was determined by comparing their intensities with the maximal intensity achieved with chemical oxidation and is shown in Table 1. Flash illumination at 532 and 730 nm light induced charge separation in nearly all PS I centers (99 and 87%, respectively). Illumination at 780 or 820 nm induced P700* in 65 and 20% of PS I, respectively.

YZ* formation in PS II was quantified by measuring the light-induced amplitude at 3445 G in Fig. 1B (indicated with *), and the fraction of YZ* formed was determined by comparison with the spectrum from a fully induced YD* (Table 1). Flash illumination at 532 nm induced YZ* oxidation in 100% of the PS II centers, whereas the yield for the far-red wavelengths was much lower. Illumination at 730 nm induced YZ* in only 39% of PS II, and no or very little oxidation of YZ was observed at 780 (2%) or 820 nm (Table 1).

**FIGURE 2.** Induction of the P700* radical EPR signal. The signal was induced at 295 K by laser flashes provided at 532 (black), 730 (red), 780 (gray), 800 nm (blue), and 820 nm (green). The flashing at 5-Hz frequency was started at t = 0 and continued until signal saturation was observed (marked with an arrow). The P700 radical EPR signal was recorded at g = 2.0046, the g-value for YZ* and YD*; and any formation of these species will therefore not contribute to the recorded EPR signal. EPR conditions were as follows: temperature, 295 K; microwave power, 8 milliwatts; modulation amplitude, 5 G; microwave frequency, 9.76 GHz a.u., arbitrary units.

**TABLE 1**

| Wavelength (nm) | P700*<sup>a,b</sup> | YZ*<sup>c</sup> |
|-----------------|---------------------|-----------------|
| 532             | 99                  | 100             |
| 730             | 87                  | 39              |
| 780             | 65                  | 2               |
| 820             | 20                  | 0               |

<sup>a</sup> The data were obtained from the EPR spectra shown in Fig. 1.

<sup>b</sup> Quantification of P700* was achieved by comparing the intensity for the light-induced P700* radical EPR spectra with the intensity achieved after complete chemical oxidation of P700 (see text).

<sup>c</sup> YZ* was quantified from the light-dark difference spectrum and compared with the dark stable YD* radical, which amounts to one radical per one PS II center (see text).
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FIGURE 3. Action spectra of P700 (filled circles, spinach; open circles, N. punctiforme) and Yz oxidation (in spinach; triangles) in the far-red region at 295 K. The formation of P700+ and Yz+ is presented as the fraction of reaction centers that was oxidized (%) at the respective excitation wavelength. For comparison, the data for Yz+ formation using single flashes in PS II-enriched membranes from our earlier publication (23) are also shown (gray circles).

no quantifiable oxidation of Yz in PS II occurred (Fig. 3). Fig. 3 also shows the action spectrum for Yz oxidation. The spectrum we show here is similar to that described earlier by us (38). It is thus clear that PS I is functional at wavelengths much higher than those used by PS II.

Formation of P700+ in the PS I Reaction Center from N. punctiforme at 295 K—We also studied induction of P700+ in purified PS I reaction centers from N. punctiforme. The data presented in Fig. 4 show the light-dark difference spectra obtained after illumination at different wavelengths. This preparation contains no PS II; consequently the EPR analysis is very straightforward, and all spectra shown represent P700+. It is clear that almost the same quantitative amount of P700+ was formed by illumination at 532 nm (spectrum a), 730 nm (spectrum b), and 780 nm (spectrum c). We also observed P700+ formation with illumination at 810 nm where the signal size was much smaller (spectrum d). We could not observe P700+ signal formation beyond 810 nm, but this might reflect the limited sample concentration available (0.035 mg of Chl/ml). At higher wavelengths, any formed signal would disappear in the noise. The g-values for all spectra were determined to be 2.0026 (indicated with dotted line), and the peak to trough width was 7 G (solid lines), allowing unambiguous assignment of this signal to P700+. The wavelength-dependent yield of P700+ in the PS I reaction center preparation (Fig. 3, open circles) is comparable with the P700+ oxidation achieved in thylakoid membranes from spinach (Fig. 3, closed circles).

Reduction of the Acceptor Side of PS I by Far-red Light at 15 K—So far we reported that the induction of P700+, the primary donor in PS I, is possible by illumination with far-red light up to 840 nm. The question is then whether stable charge separation is achieved and which acceptor in PS I is reduced after illumination at these far-red wavelengths. The stability of the P700 radical is a good indication that stable charge separation indeed occurs, but direct observation of reduced cofactor(s) from the acceptor side of PS I would cast further light on the far-red photochemistry in PS I.

The reduction of the terminal acceptor in PS I, iron-sulfur center Fb, takes place in >500 ns at room temperature (9, 10) and is followed by the reduction of ferredoxin in the microsecond time range (9, 10, 54). This is too fast to be kinetically observed by conventional EPR spectroscopy. However, it is possible to induce stable charge separation in PS I by illumination at liquid helium temperatures where both of the Fα and Fβ iron-sulfur centers become visible by EPR in their reduced form. Illumination of PS I at 15 K normally results in a mixture of PS I centers with either Fα− or Fβ− present (55). Both of them are easily recognizable as g = 2.0026 radical EPR signals in light-dark difference spectra.

Such difference EPR spectra obtained after illumination of intact thylakoid membranes at 15 K by laser flashes at 532 nm (spectrum a), 730 nm (spectrum b), 780 nm (spectrum c), 800 nm (spectrum d), and 820 nm (spectrum e) are shown in Fig. 5a. Illumination with 532 nm light induced a maximal signal from the reduced Fα and Fβ iron-sulfur centers. In our measuring conditions, all gα, gγ, and gδ regions are clearly observed for both EPR species (spectrum a). Illumination at 730 and 780 nm induced 75 and 40% of the signal, respectively (spectra b and c). 17% of the signal was induced by light at 800 nm. Above 800 nm, less than 10% of the signal from Fα and Fβ acceptors was visible (spectrum e). The Fα/Fβ reduction ratio was similar after illumination at all wavelengths irrespective of total signal size as can be seen by comparing the intensity of the shoulder (Fβ center) and the peak (Fα center) at the gγ region (Fig. 5a, marked with *).

Fig. 5b shows the reduction of Fα and Fβ acceptors as a function of flash number at different wavelengths. At 532 and 730 nm, the reduction was fast and efficient, and after only 5 min of flashing, the reduction was complete. Much slower and also less efficient induction occurred at 780, 800, and 820 nm where it took ~30 min of flash illumination to reach a stable level of reduction at 15 K (Fig. 5b). The yield of the iron-sulfur center reduction at 15 K was lower as compared with the fraction of P700+ oxidation at 295 K (Figs. 1 and 2). The reason is that stable...
charge separation involving $F_A/F_B$ reduction is known to occur only in a fraction of PS I at low temperatures (35–50% below 100 K) due to competing recombination reactions (56).

The action spectrum of the $F_A$ and $F_B$ reduction at 15 K is shown in Fig. 5C. All data points were taken at the same power of excitation laser flashes (20 mJ) at different wavelengths. Reduction of both acceptors is clearly observable up to 830–840 nm. The shape of the action spectra obtained at 15 K is somewhat different from that obtained at 295 K with the half-induction point seemingly shifted to lower wavelengths. However, it is clear that low temperature photochemistry and reduction of the acceptor side in PS I both are functional up to 840 nm. This is similar to the far-red limit observed in the room temperature measurements.

**DISCUSSION**

Simultaneous Detection of PS I and PS II Photochemistry Induced by Far-red Light—The far-red limit for PS II-induced photochemistry at 295 K was recently demonstrated to be at 810 nm (38), 130 nm above the absorption maximum of the primary donor $P_{680}$. This triggers the question whether other photosynthetic reaction centers also can perform charge separation and secondary electron transfer at wavelengths far above the main absorption of the primary donor.

In this study, we investigated the far-red limit and efficiency for oxidation of $P_{700}$, the primary donor in PS I. PS I-induced photochemistry was earlier reported at 760 nm in *Synechococcus elongatus* (53). In contrast to this, we show that, in thylakoid membranes from spinach where both PS I and PS II are present together with their respective large membrane-bound antenna systems, $P_{700}^{+}$ can be formed in almost all PS I centers when excitation light up to 780 nm. Furthermore, a substantial amount of $P_{700}$ oxidation was also observed above 820 nm (Fig. 1). This demonstrates that the limit for PS I-induced photochemistry has to be reconsidered (53).

Our results also clearly show that $P_{700}$ can be oxidized by far-red light to the same extent and in the same wavelength range in a purified PS I reaction center from the cyanobacterium *N. punctiforme* (Fig. 4). This is interesting and clearly shows that the outer membrane-bound antenna in spinach (higher plants) is not vital for far-red light-induced photochemistry in PS I. It also shows that far-red light is able to induce PS I-related photochemistry in the same way in different species.

Because our spinach experiments were performed in Tris-washed thylakoid membranes, it also was possible to reinvestigate the PS II-induced photochemistry in the far-red, measured synchronously in the same samples by detection of the $Y_{Z}^{+}$ radical (Fig. 1B). It is clear that $Y_{Z}^{+}$ has a narrower induction range than $P_{700}^{+}$, and very little or no $Y_{Z}^{+}$ was detected during illumination with flashes >785 nm (Fig. 4). This result is in good agreement with our previous measurements of $Y_{Z}^{+}$ in PS II-enriched membranes (38). In our earlier work, the far-red limit for light-induced electron transfer in PS II was determined to be 810 nm by detection of the variable fluorescence formed after photoaccumulation (38). This is 25 nm beyond the detection limit of $Y_{Z}^{+}$ (Fig. 4 in Ref. 38). The reason for this difference is the relatively short life time of $Y_{Z}^{+}$ also in manganese-depleted thylakoid membranes. Together with weak absorption in this part of the spectrum, this makes it problematic to induce quantifiable amounts of the transient $Y_{Z}^{+}$ radical above 780 nm, although charge separation and oxidation of the CaMn$_{4}$ cluster occur and can be quantified in a photoaccumulation measurement using intact PS II preparations.

The Far-red Limit of PS I—It is also interesting to determine exactly where the limit of PS I photochemistry is. We therefore determined the action spectra of the maximum inducible amount of $P_{700}^{+}$ and $Y_{Z}^{+}$ using excitation wavelengths ranging up to 840 nm (Fig. 3). Clearly PS I-induced photochemistry occurred with excitation light up to 840 nm, and close to max-

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**FIGURE 5.** $A$, reduction of the $F_A$ and $F_B$ iron-sulfur centers in PS I in thylakoid membranes from spinach at 15 K induced with laser flashes provided at the following different excitation wavelengths: 532 (spectrum a), 730 (spectrum b), 780 (spectrum c), 800 (spectrum d), and 820 nm (spectrum e). The flashing was continued until saturation of the signal was achieved at the respective wavelength. The spectra are light-dark difference spectra and show superposition of both $F_A^{+}$ and $F_B^{+}$ centers. The * indicates the peaks of the $g_r$ region that were used for quantification. The large absorption of the primary donor in PS I. **B**, time course of formation of the $F_A^{+}$ and $F_B^{+}$ iron-sulfur centers after illumination at 15 K by laser flashes provided at 532 (black), 730 (red), 780 (gray), 800 (blue), and 820 nm (green). The flashing at 5-Hz frequency was started at $t = 0$ (marked with an arrow) and continued until signal saturation was observed. The signal amplitude was estimated from the peak in the $g_r$ region marked with * in A. **C**, action spectrum of the reduction of the $F_A$ and $F_B$ iron-sulfur centers in the far-red region at 15 K. The data are from the measurements shown in A. The formation of $F_A^{+}$ and $F_B^{+}$ is presented in percentage of the maximal induction of the signals obtained by illumination at 532 nm. a.u., arbitrary units.
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imum induction was seen as high as 780 nm in intact PS I complexes from spinach. Importantly, the action spectrum is similar in purified PS I reaction centers from *N. punctiforme*. This shows that charge separation can be driven with light 140 nm above the absorption peak at 700 nm for P700 and more than 100 nm above the absorption band of the so-called red Chls (see below) (31). Our results also extend the action spectrum of PS I 80 nm further out to the red than reported previously (53).

The P700**+** radical decays slowly after a flash and is stable in chemically oxidized PS I preparations. Therefore, in our experiment where we used continuous flashing, any formed P700**+** has been accumulated. Almost all PS I reaction centers were oxidized up to 780 nm in both the reaction center preparation from *N. punctiforme* and the spinach thylakoid membranes. In both preparations, the oxidation of P700 started to decrease only above 780 nm following a quite sharp far-red edge profile. Therefore, we conclude that the decrease of P700 oxidation above 780 nm is probably coupled to intrinsic properties in the PS I reaction center core instead of any properties in the outer antenna complexes, which are absent in the reaction center preparation.

The sharp edge profile of the far-red limit in the action spectrum of PS I between 810 and 830 nm where almost 50% of the P700**+** oxidation was lost (Fig. 3) is an interesting observation. Our hypothesis is that this reflects the presence of a narrow absorption band in this region giving rise to charge separation and electron transfer. The nature of this band is not clear, but it most likely originates from a single absorbing entity involving either a monomeric chromophore or dimeric coupled chromophores (see below). The energetic limitations are most likely negligible because the difference in photon energy between 810 and 830 nm light is less than 3%.

It is also interesting to compare the P700**+** formation at 295 K (Fig. 3) with the F_A and F_B reduction at 15 K (Fig. 5C). The two measurements represent products of the same stabilized charge separation in PS I; however, they are performed at very different temperatures. Despite this, they exhibit very similar far-red edge limitation in the respective action spectra. It is thus clear that charge separation in PS I is functional at both temperatures up to approximately 840 nm (Figs. 3 and 5C). This indicates that very little or no thermal activation is required for this reaction in the far-red region.

Possible Identity of the Far-red Light-absorbing Pigments—

We report here that charge separation in PS I is formed by light up to 840 nm. This is far above where most species involved in PS I photochemistry normally are considered to absorb light. It is therefore interesting to discuss the identity and location of potential photosynthetic chromatophore candidates for the absorption in the far-red light and the subsequent energy transfer to P700**+**.

Several of these chromatophores and antenna complexes can be ruled out as responsible for the far-red absorption leading to the charge separation seen in the present study. This holds for the pigments associated with the phycobilisomes that are not present in higher plants and are also not present in the purified PS I reaction center from *N. punctiforme*. In addition, the observation of far-red photochemistry in PS I from a cyanobacterium also seems to exclude pigments in the extrinsic mem-
brane-bound LHC I antenna in higher plants. This is particularly important because this antenna system contains red-shifted Chls that absorb light above 700 nm (23–25). They could have been obvious candidates for the far-red-absorbing pigments giving rise to the far-red photochemistry, but because they are not present in cyanobacteria, we argue that the red Chls in LHC I do not drive the photochemistry described here.

The same kind of argument can be used to eliminate another class of far-red-absorbing Chls sometimes proposed to be formed by interacting Chls in the interface between the PS I monomers in the trimeric PS I reaction centers in cyanobacteria. These trimers are not present in higher plants where PS I is monomeric in nature. From these arguments, we conclude that it is unlikely that the species responsible for the far-red absorption and energy transfer to P700 are found in the extrinsic antenna systems or at the interface between the reaction centers in the trimeric PS I in cyanobacteria.

Instead we propose that the far-red-absorbing species should be sought among the pigments in the PsaA/PsaB heterodimer, which is very similar between the thylakoid membranes in spinach and the PS I reaction center preparation from *N. punctiforme*, i.e. plants and cyanobacteria. There are many pigments bound to the PsaA/PsaB heterodimer, and it is at this stage impossible to identify which of them are responsible for absorption in the far-red region.

However, useful analogies can be drawn with the PS II reaction center. The central part of PS II is composed of the D1/D2 reaction center that can be prepared without other pigment-containing subunits. The D1/D2 reaction center contains only eight tetrapyrrole molecules, two phaeophytin *a* molecules, and six Chl *a* molecules and is able to perform charge separation, whereas the secondary electron transfer is very limited. These pigments are tightly packed, and at least four of them are coupled to each other (57). This packing of close lying pigments gives rise to electronic and structural configurations where these pigments are excitonically coupled and gives rise to at least one identified charge transfer band in the far-red region (35). This band has absorption up to 730 nm at 5 K (35, 36, 58). Other, hitherto undiscovered charge transfer bands even further out in the far-red part of the spectrum possibly also exist. There are several indications that such a state might drive PS II photochemistry observed up to 810 nm at 295 K (38), and we have recently found that far-red light can drive both primary and secondary electron transfer in PS II up to at least 750 nm also at 5 K (41).

The structural analogies between the central parts of PS I and PS II are large (59). The central pigment molecules in the PS II reaction center are not too differently organized compared with the six Chl *a* molecules forming the central part of PS I (Scheme 1). In both reaction centers, porphyrin pigments are closely located and show excitonic coupling and the presence of charge transfer states (32, 36, 60). These features are analogous to the red-shifted Chls in LHC I (see above). Coupled pigments can have the ability to absorb light of much lower energy than the monomeric pigment and can therefore be excited with far-red light (61). It is thus not improbable that charge transfer bands in the far-red can exist also in the PS I reaction center.
If a charge transfer state absorbing weakly in the far-red is able to drive photochemistry and charge separation in the PS II reaction center there is no reason why a similar state, if it exists, should not be able to do so also in PS I. Our hypothesis to explain our results here is that one or several such weakly absorbing charge transfer states exists in PS I. The question is then where, within the six Chlα molecules in the PS I reaction center (Scheme 1), can charge transfer band(s) be located.

Again, useful analogies with the PS II reaction center and the reaction center from purple bacteria can be drawn. The special pair in the reaction center from purple bacteria exhibits strong absorption at 800 nm (37). The proposed charge transfer free energies is responsible for the smaller number of PS I reaction centers in which resonant excitation can occur at higher wavelength, i.e. at 820 nm (63). At lower wavelength (i.e. at 730 nm), the charge transfer state is mixed with the excitonic states of the red Chls and results in photochemistry in almost 100% of the PS I centers.

Our results are in line with this model, and our experiments extend the observed far-red photochemistry phenomenon in PS I to 840 nm to another cyanobacterium (N. punctiforme) and to higher plants. At present, the pigment species absorbing this far-red light are not known. The absorption is very low, and their identification will be difficult among the very many Chl molecules present in any PS I reaction center preparation. Therefore, their identification will require further spectroscopic investigations, but also theoretical analysis combined with site-directed mutagenesis modifying the environment of candidate Chls will most likely prove essential for their identification. The same holds for the very interesting possibility that a charge transfer state of this kind might in fact be preferentially connected to one of the electron transfer branches in PS I (A or B branch). The analogous charge transfer state in PS II driven by far-red light at 5 K is only active in the Yz/CaMn4 cluster pathway, whereas it is hardly connected to the Chlβ-carotene/cytochrome b559 pathway (41). Thus, there is precedence for asymmetric electron transfer between visible and far-red light in PS II.

An interesting aspect is whether the far-red-driven electron transfer has any role to fulfill in natural photosynthesis outside the laboratory. The absorption is very low, and the far-red photosynthesis will never promote large rates in environments where normal daylight is available. However, an organism may find itself for shorter or longer times in a habitat in which white light is lacking but far-red light is available. In such an environment, far-red photosynthesis, if it were the only energy source for the organism, also could play a significant role.

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Far-red Limit of Photosystem I

The light absorption in the far-red region was also estimated. By using highly concentrated purified PS I core preparations, a weak and featureless absorption that extends to 850 nm was observed (63). The absorption above 750 nm was less than 0.1% of the absorption at the maximum in the Qb region at 680 nm (63). The actual value could be even lesser due to light scattering and/or baseline correction interferences. In any case, this low absorption alone cannot account for the quite efficient charge separation observed here and in Schlodder et al. (63) in the 750 – 840-nm region (20% of the P700 + formation at 820 nm at 297 K; Figs. 1 and 2).

The questions then arise how the proposed charge transfer state can contribute to such a high efficiency and why this efficiency drops with increasing wavelength? The model proposed by Schlodder et al. (63) satisfactorily addresses these questions. The proposed charge transfer state is of dipolar nature and exhibits large inhomogeneous broadening. The smaller distribution of the charge transfer free energies is responsible for the smaller number of PS I reaction centers in which resonant excitation can occur at higher wavelength, i.e. at 820 nm (63). At lower wavelength (i.e. at 730 nm), the charge transfer state is mixed with the excitonic states of the red Chls and results in photochemistry in almost 100% of the PS I centers.
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