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Primary Photochemistry of Photosystem II of Photosynthesis

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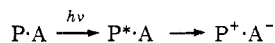
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The heart of photosynthesis resides in the primary photochemical reactions which convert light energy into the chemical free energy needed to transform carbon dioxide and water into carbohydrate. This Account examines these photochemical reactions with particular emphasis on recent work on photosystem II (PSII) of green plants.

The photosynthetic apparatus is organized into units with several hundred chlorophyll molecules which absorb light energy and transfer this energy to a special chlorophyll complex (usually denoted P with the appropriate notation for its absorption maximum) associated with the reaction center of the unit. The excited reaction center chlorophyll transfers an electron to an electron-acceptor molecule, A, complexed to the reaction center.¹ The production of the primary oxidant, P⁺, and the primary reductant, A⁻, provides the free energy needed to drive the dark reactions of photosynthesis.

Much of our understanding of the primary photochemical mechanisms of photosynthesis has come from investigations of photosynthetic bacteria. One experimental approach has been to correlate absorbance changes of the reaction center chlorophyll (oxidation of P₈₇₀ causes it to bleach) with fluorescence yield changes of the light-harvesting chlorophyll.^{2,3} Light energy absorbed by the bulk chlorophyll travels through the photosynthetic unit as excitons until it is reemitted as fluorescence, is converted to heat, or arrives at a reaction center. If the reaction center is open, the P·A state, the energy is rapidly processed to chemical free energy.



To the extent that reaction centers are closed because the reaction center chlorophyll is bleached, the P⁺·A⁻ and P⁺·A states, the energy cannot be extracted from the light-harvesting chlorophyll and the fluorescence yield increases.^{2,3} It is also possible that the reaction center cannot process the energy because the electron acceptor was already reduced, the

P·A⁻ state. In that case the excitation energy appears to be transferred back to the bulk chlorophyll where it may be emitted as fluorescence.³ In bacterial systems, either the oxidation of P or the reduction of A causes the fluorescence yield to increase.

The analysis of fluorescence yield changes generally assumes that the fluorescence from the reaction center chlorophyll is negligible in comparison to that from the bulk chlorophyll. In the case of bacterial systems where the reaction centers can be extracted and studied apart from the rest of the photosynthetic apparatus, the reaction center chlorophyll has been shown to have a very low fluorescence yield⁴ so that the assumption appears valid. In the case of green plants the same assumption is generally made, but it is not so well established.

Photosynthesis is more complex in green plants than it is in bacteria in that two photochemical pigment systems, PSI and PSII, are involved (Figure 1). Absorption of light by the bulk chlorophyll of PSI units results in the oxidation of P₇₀₀ and the reduction of an unknown primary electron acceptor, X. Recent epr measurements at 25°K suggests that X may be a bound form of ferredoxin.^{5,6} Light-induced absorbance changes at 430 nm⁷ also have been related to the primary electron acceptor of PSI. The photo-oxidation of P₇₀₀ can be followed by its absorbance change but (for unknown reasons) fluorescence yield changes do not accompany the primary photochemistry of PSI.

Absorption of light by PSII units results in the formation of P₆₈₀⁺ and Q⁻. However, the absorbance changes of P₆₈₀ are small and very short-lived and are difficult to measure in the presence of the large background absorbance at 680 nm from the bulk chlorophyll. Also, the small absorbance changes are difficult to distinguish from fluorescence yield changes which accompany the reaction.⁸ All fluores-

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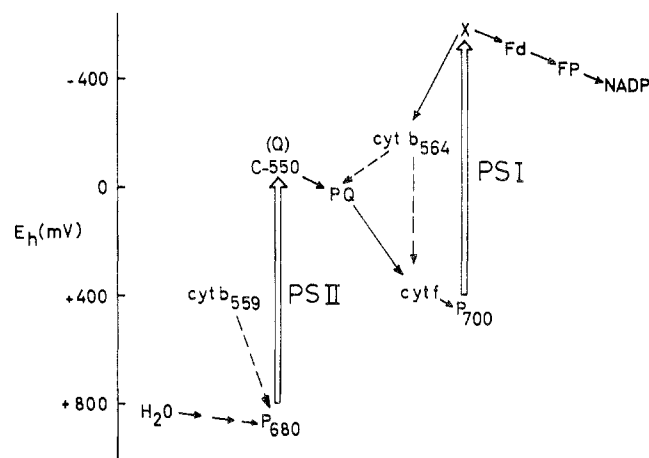


Figure 1. Photosynthetic electron-transport scheme for green plants. Dashed arrows indicate uncertain pathways. Fd indicates ferredoxin and FP a flavoprotein.

cence yield changes in green plants are ascribed to PSII activity and have been related to the primary electron acceptor, denoted Q for its fluorescence quenching properties.⁹ When Q is in the oxidized state excitation energy is used to reduce Q and the fluorescence yield is low; when Q is in the reduced state the fluorescence yield increases because the excitation energy cannot be processed. Direct correlations between fluorescence yield changes and the bleaching of P₆₈₀ have not yet been made with chloroplasts because of the difficulties in making the P₆₈₀ measurements, but it has generally been assumed that the fluorescence yield of chloroplasts is determined solely by Q. (This statement refers only to the correlation between fluorescence yield and the primary photochemistry. Secondary biochemical processes which modify the "energy state" of the thylakoid membranes also affect fluorescence yield¹⁰ but apparently without altering the redox state of Q.) By analogy with bacterial reaction centers, however, the fluorescence yield of chlorophyll should be determined by the redox states of both P₆₈₀ and Q. Recent evidence that the redox state of the primary electron donor to PSII does affect fluorescence yield will be discussed in this article.

A new tool for the study of the primary photochemistry of photosynthesis in green plants was provided by Knaff and Arnon¹¹ in their discovery of a light-induced absorbance change near 550 nm which they attributed to an electron-transfer component, C-550. Evidence relating C-550 to the primary electron acceptor of PSII is reviewed in the first part of this Account, and examples are presented of what has been learned from using the C-550 measurement as an assay for the primary photochemical activity of PSII. The final part of this Account considers the relationship of fluorescence yield to the primary photochemical activity of PSII with emphasis on cases where fluorescence yield does not reflect the redox state of the primary acceptor.

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The Measurement of C-550

Knaff and Arnon first observed C-550 as a light-induced bleaching at 550 nm relative to 540 nm in spinach chloroplasts.¹¹ Ferricyanide was added to the chloroplasts to oxidize the cytochrome *f* which would otherwise obscure the light-induced absorbance changes of C-550. Knaff and Arnon reported that this component was not a cytochrome on the basis that no corresponding Soret band was found.¹¹ They also showed that irradiation of chloroplasts at liquid nitrogen temperature resulted in the bleaching of C-550 at 546 nm and the oxidation of cytochrome *b*₅₅₉.¹² The low-temperature photoreactions were confirmed in several laboratories.¹³⁻¹⁵ The light-induced absorbance change of C-550, which involved an absorbance increase at 543 nm as well as a bleaching at 546 nm (at -196°), was shown to accompany a chemical reduction which could be achieved in the dark with strong reductants such as dithionite.¹³ The biphasic absorbance change was attributed to a shift of the absorption maximum of C-550 from 546 nm in the oxidized form to 543 nm in the reduced form.¹⁶ Both the photoreduction of C-550 and the photooxidation of cytochrome *b*₅₅₉ were ascribed to PSII activity on the basis that red light was more effective than far-red light^{11,14} and that photosynthetic systems which lack PSII activity, such as PSI subchloroplast particles and certain photosynthetic mutant strains, do not show either of the photoreactions.¹³

Absorption spectra of spinach chloroplasts at -196° before and after a 30-sec irradiation with red light are shown as curves D and L, respectively, in Figure 2. The light-minus-dark difference spectrum (L - D) was plotted at a fourfold increase of sensitivity. The bleaching at 546 nm and the absorbance increase at 543 nm in the difference spectrum are due to the reduction of C-550 and the bleaching at 556 nm is due to the oxidation of cytochrome *b*₅₅₉. The absorption bands of cytochrome *f* and cytochrome *b*₅₅₉ can be seen on close examination of the absolute spectra. Cytochrome *f* is not affected by irradiation at low temperature. The absorption bands of C-550 are not so readily discerned in the absolute spectra although the shift is readily apparent.

However, the absolute absorption bands of oxidized and reduced C-550 can be resolved by fourth derivative spectroscopy.^{17,18} The fourth derivative curves of the absolute spectra are presented in Figure 2 as D^{IV} and L^{IV}. The fourth derivative bands at 548 and 552 nm represent the absorption maxima of the split α band of cytochrome *f* and the band at 556 nm is due to cytochrome *b*₅₅₉. The fourth derivative band of oxidized C-550 (at 546 nm) is masked by the

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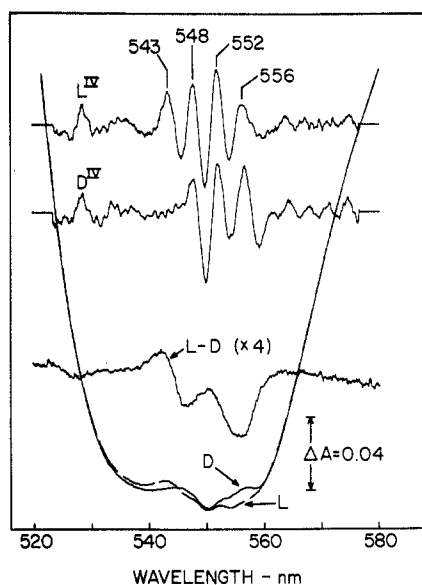


Figure 2. Absolute absorption spectra of chloroplasts at -196° before (curve D) and after (curve L) a 30-sec irradiation with red light: the difference spectrum (L - D) between the irradiated and unirradiated samples at a fourfold increase of sensitivity and the fourth derivative curves of the absorption spectra of the irradiated and unirradiated samples, L^{IV} and D^{IV} , respectively.

548-nm band in D^{IV} , but the reduced band at 543 nm is readily apparent in L^{IV} . In the presence of ferricyanide, added to oxidize cytochrome *f* and cytochrome b_{559} prior to freezing to -196° , the fourth derivative band of C-550 is observed at 546 nm before irradiation and at 543 nm after irradiation or chemical reduction.^{19,20}

C-550 as the Primary Electron Acceptor of PSII

The observation that C-550 could be chemically reduced by dithionite¹³ suggested that it might be related to the fluorescence quencher, Q, since Q showed similar redox properties.²¹ Redox titration experiments were made to compare the photoreduction of C-550, the photooxidation of cytochrome b_{559} , and the light-induced fluorescence yield change.²² In these experiments the chloroplasts were suspended in an anaerobic mixture of redox buffers, the redox potential was established at 0° , and samples were frozen to -196° . The absorption spectrum and the relative fluorescence yield of the samples were measured before and after irradiation at -196° . Figure 3 shows a semilogarithmic plot of the light-induced changes of C-550, cytochrome b_{559} , and fluorescence yield as a function of the redox potential at which the samples were frozen. All three of the light-induced changes followed the same titration curve which was very close to the curve for a one-electron Nernst equation with a midpoint potential of +25 mV.

The close agreement between the titration curves for C-550 and fluorescence yield supports the correlation between C-550 and Q. The correspondence be-

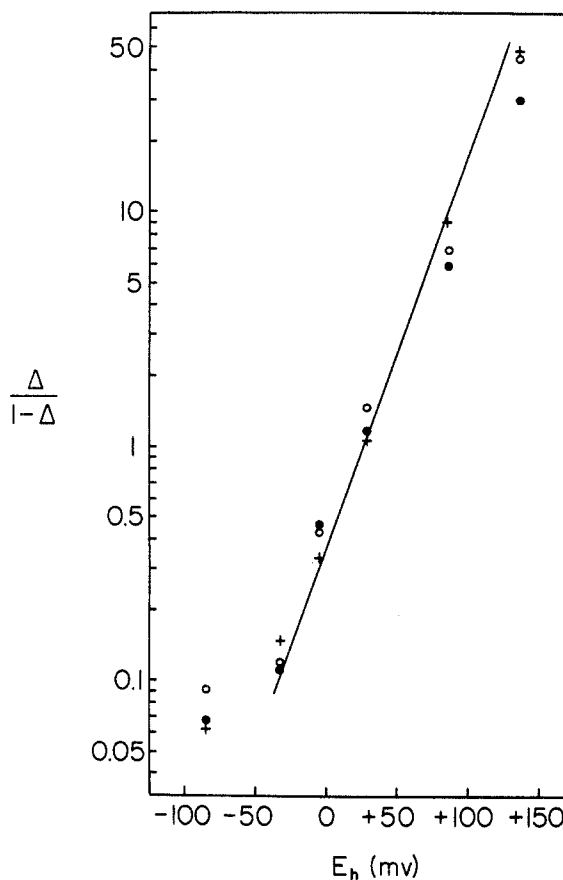
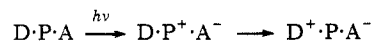


Figure 3. Semilog plot of $\Delta/(1 - \Delta)$ where Δ is the fraction of the component available for the light-induced absorbance change at -196° as a function of the redox potential of a suspension of chloroplast fragments established at 0° prior to freezing. Light-induced absorbance changes at 546 nm (O) and 556 nm (●) and the light-induced fluorescence yield changes (+) are shown. $\log [\Delta/(1 - \Delta)]$ is equivalent to $\log [(C-550 \text{ oxidized})/(C-550 \text{ reduced})]$. The straight line in the semilog plot of the Nernst equation represents a one-electron transition with a midpoint potential of +25 mV. The best fit to the experimental data indicates a 0.98-electron reaction.

tween the titration curves for cytochrome b_{559} and C-550 shows that the photooxidation of cytochrome b_{559} at -196° requires C-550 to function as an electron acceptor. This is taken as evidence that C-550 is the primary electron acceptor of PSII. (The question of whether C-550 is the actual electron acceptor or an isomorphous indicator of the redox state of the acceptor is discussed later.) Cytochrome b_{559} is not considered to be the primary electron donor since chemical oxidation of the cytochrome does not prevent the photoreduction of C-550. (The term "primary" is used to indicate reactions which are a direct consequence of the absorption of light.) The photoreactions at -196° are consistent with a simple model for the PSII reaction center



where D is cytochrome b_{559} , A is C-550, and P is the reaction center chlorophyll of PSII, P_{680} .

A variety of other experiments were designed to try to find conditions where the redox state of C-550 and Q were not equivalent. The success of any such experiment would indicate that C-550 was different from Q. (1) Mutants of *Scenedesmus*¹³ and *Chlamydomonas*¹⁹ which have a high invariant fluorescence yield, as if they lacked quencher Q, had no

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(20) B. L. Epel, W. L. Butler, and R. P. Levine, *Biochim. Biophys. Acta*, **275**, 395 (1972).

(21) W. A. Cramer and W. L. Butler, *Biochim. Biophys. Acta*, **172**, 503 (1969).

(22) K. Erixon and W. L. Butler, *Biochim. Biophys. Acta*, **234**, 381 (1971).

C-550; the high potential cytochrome b_{559} was also absent. (2) Normal chloroplasts were irradiated with red light during the cooling to freeze in the high fluorescence yield condition. In this case C-550 was in the reduced state in the frozen chloroplasts, as was the cytochrome b_{559} . Irradiating these chloroplasts at -196° had no further effect on fluorescence yield, C-550, or cytochrome b_{559} .²² (3) A high fluorescence yield in the dark was achieved by irradiating chloroplasts in the presence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) and hydroxylamine. In the presence of these inhibitors the fluorescence yield remains high after the light is turned off.²³ C-550 also remained in the fully reduced state in the dark after the irradiation.²² In all of these experiments a close correspondence between C-550 and Q was indicated.

The correspondence between C-550 and Q, which is well supported by measurements of the light-induced absorbance and fluorescence yield changes at low temperature, is not so apparent in room temperature measurements. In fact, Ben-Hayyim and Malkin concluded on the basis of room temperature measurements that C-550 bore no relationship to Q.²⁴ This discrepancy was apparently resolved, however, by results which indicated that approximately half of the absorbance change attributed to C-550 at room temperature resulted from light-induced changes of membrane potential.²⁵ Changes in the energy state of the chloroplast membranes at room temperature can modify the measurements of both C-550 and fluorescence yield in directions which tend to offset the positive correlation resulting from the primary photochemical changes.²⁵

C-550 as an Assay for PSII Activity

The photoreduction of C-550 at low temperature provides an assay which isolates the primary photochemical reactions associated with the PSII reaction centers from the subsequent electron-transport reactions. This assay can be used to determine whether a specific inhibitory treatment attacks the reaction centers directly or inhibits a dark reaction outside of the reaction center. For instance, inhibition of electron transport by adding DCMU or by a Tris-washing treatment has no effect on the photoreduction of C-550 at -196° . One would expect that the Tris-washing treatment which inhibits electron transport between water and PSII²⁶ would not inactivate the PSII reaction centers since electron transport through PSII is restored by adding compounds which supply electrons to PSII.^{26,27} DCMU, however, acts very close to the PSII reaction centers. Fluorescence data have been interpreted to indicate that DCMU does not prevent the photoreduction of the primary electron acceptor but does block electron transport to the next electron carrier.⁹ Döring, *et al.*,²⁸ and

Siggel, *et al.*,²⁹ however, have proposed that DCMU reacts directly with the reaction center chlorophyll and inhibits the primary photochemistry of PSII. The observations that the photoreduction of C-550, the photooxidation of cytochrome b_{559} , and the fluorescence yield change occur at -196° in the presence of DCMU would argue against their proposal.

The assay method has also been used to examine inhibition by lipase, proteinase, and ultraviolet radiation. Prolonged incubation of chloroplasts with pancreatic lipase³⁰ or trypsin³¹ or relatively large doses of uv radiation³² result in the loss of the light-induced absorbance changes at low temperature. Thus, these treatments appear to attack the reaction centers directly. Lower doses of uv radiation³³ and probably shorter incubation times with lipase or trypsin inhibit oxygen evolution between water and PSII without inactivating the reaction center.

The absence of the absorbance changes after prolonged lipase treatment was shown to be due to the actual destruction of C-550. The absorption bands of C-550 were absent after the lipase treatment so that the absolute absorption bands of the oxidized and reduced forms of C-550 could be measured in difference spectra between normal and lipase-treated chloroplasts.¹⁶ The absence of C-550 after lipase treatment could also be shown by fourth derivative spectra of the treated chloroplasts.

The measurements of light-induced absorbance changes of C-550 at low temperature were used to determine if the PSII activity could be removed by organic solvent extraction and replaced by reconstitution with known compounds.³⁴⁻³⁶ Lyophilized chloroplasts showed a normal photoreduction of C-550 and photooxidation of cytochrome b_{559} . Extracting the chloroplasts six times with hexane eliminated most of these changes. Reconstituting the chloroplasts with the crude extract or with a mixture of pure β -carotene and plastoquinone A, PQA, restored both photoinduced changes. Reconstitution with β -carotene alone restored the C-550 change but not the cytochrome b_{559} change. Other data showed that cytochrome b_{559} had been modified to a lower potential, autoxidized form by the extraction treatment and thus was already oxidized in the β -carotene reconstituted chloroplasts. When the β -carotene reconstituted chloroplasts were suspended in a buffer containing ascorbate before freezing, the cytochrome b_{559} was in the reduced state and was photooxidized when C-550 was photoreduced.³⁴ It should also be noted that Strichartz found that the 518-nm absorbance change in spinach chloroplasts was removed by extraction with heptane and partially restored by reconstitution with β -carotene.³⁷

Oxygen evolution in a ferricyanide Hill reaction

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 (30) S. Okayama, B. L. Epel, K. Erixon, R. Lozier, and W. L. Butler, *Biochim. Biophys. Acta*, **253**, 476 (1971).
 (31) S. Okayama and W. L. Butler, unpublished results.
 (32) K. Erixon and W. L. Butler, *Biochim. Biophys. Acta*, **253**, 483 (1971).
 (33) T. Yamashita and W. L. Butler, *Plant Physiol.*, **43**, 2037 (1968).
 (34) S. Okayama and W. L. Butler, *Plant Physiol.*, **49**, 769 (1972).
 (35) W. L. Butler, K. Erixon, and S. Okayama in ref 24, p 73.
 (36) D. Sofrova and D. S. Bendall in ref 24, p 561.
 (37) G. R. Strichartz, *Plant Physiol.*, **48**, 553 (1971).

was also destroyed by the hexane extraction and was restored to about 50% by reconstitution with either the crude extract or with a mixture of pure β -carotene and PQA. Both compounds were needed, however. Reconstitution with β -carotene alone had no effect, and reconstitution with PQA alone had only a small effect in restoring oxygen evolution.³⁴ These experiments indicate that β -carotene was necessary and sufficient to restore C-550 and the primary photochemistry of the PSII reaction centers and that PQA was needed in addition for electron transport between water and ferricyanide.

The effects of extraction and reconstitution on fluorescence yield at -196° also supported the correlation between C-550 and Q.³⁴ The fluorescence of variable yield was removed by the extraction and was partially restored with β -carotene alone (the quenching was somewhat less after reconstitution than before extraction). PQA acted as a nonspecific quencher of both PSII and PSI fluorescence, but the presence of C-550 appeared to protect against the nonspecific quenching by PQA.

A small amount of the β -carotene in spinach leaves is present as a carotenoid-protein complex which has an absorption band at about 550 nm at room temperature.³⁴ This carotenoprotein, when purified, does not exhibit any absorbance changes on addition of dithionite or ferricyanide but its presence, in light of the requirement of β -carotene for the reconstitution of C-550 in extracted chloroplasts, is suggestive that C-550 is related to a carotenoprotein.

C-550 in Blue-Green and Red Algae

C-550 has been studied principally in higher plants and in green algae. Bendall and Sofrova reported that they could not find C-550 in the blue-green algae, *Plectonema boryanum*, and suggested that this component might not be present in blue-green algae.¹⁵ However, we have observed a normal photoreduction of C-550 and photooxidation of cytochrome b_{559} in *Cyanophora paradoxa*, a colorless dinoflagellate with blue-green algae inclusions.³⁸ These absorbance changes were also detected in the blue-green alga, *Anacistis marina*, and the red alga, *Porphyidium aeruginum*, but at lower levels than were observed in spinach chloroplasts.³⁸ Absorbance by the phycobilin pigments interferes with these measurements in the red and blue-green algae, but there is no doubt that C-550 is present and functional in these algae.

The Nature of C-550

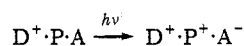
The question of whether C-550 is a specific chemical compound or is a manifestation of a structural complex is open. The available data on the extraction and reconstitution of chloroplasts are consistent with C-550 being a part of a structural complex involving β -carotene. In fact, C-550 could be a manifestation of an active PSII reaction center. If so, it may not be possible to extract C-550 as a specific redox compound which can be characterized chemically. Similar considerations also apply to other components such as P_{700} which are defined in part by their functionality in an organized complex.

Nor can we decide whether C-550 is the actual primary electron acceptor of PSII or only an indicator of the redox state of the primary acceptor. The small absorption band shift of C-550 on reduction appears more like an indicator response than a redox change. If C-550 is a carotenoprotein which is a part of the PSII reaction center complex, reduction of the primary electron acceptor might exert an electric field or other influence to shift the C-550 absorption band. If C-550 is such an indicator, however, it senses chemical reduction as well as photochemical reduction. By analogy, the P_{800} pigment of bacterial reaction centers appears to indicate the redox state of P_{870} by shifting its absorption band slightly to shorter wavelength when P_{870} is oxidized either chemically or photochemically. However, the distinction between the actual acceptor and an isomorphous indicator of the acceptor remains largely a semantic one until experimental means of separating the two activities are found. In the absence of such experiments it seems reasonable to consider C-550 to be the actual electron acceptor from an operational point of view.

The Oxidizing Side of PSII

The correlation of C-550 with the primary electron acceptor of PSII has provided an important tool to explore the primary photochemistry of photosynthesis. The close correspondence between absorbance changes of C-550 and fluorescence yield changes showed that C-550 was equivalent to Q under a wide variety of experimental conditions. However, conditions were found where fluorescence yield changes did not correlate precisely with C-550 changes, and experiments under these conditions have further elucidated the primary photochemical reactions of PSII.

It was found that the magnitude of the light-induced fluorescence yield increase at -196° depended on the redox state of the chloroplasts at the time of freezing.³⁹ Under normal conditions when cytochrome b_{559} was in the reduced state irradiation of dark adapted chloroplasts at -196° caused the fluorescence yield to increase about fivefold above the initial F_0 level. However, if ferricyanide was added prior to freezing, the fluorescence yield increased only twofold from the same F_0 level. Redox titration experiments showed that the extent of the fluorescence increase depended on the amount of cytochrome b_{559} present in the reduced form at the onset of irradiation at -196° . It was postulated that when cytochrome b_{559} was initially in the oxidized state the photoreaction at low temperature might result in the stabilization of P^+



in which case the low fluorescence yield could be due to quenching by P^+ . We have not been able to confirm the stabilization of P_{680}^+ in the reaction centers, and alternative explanations for the quenching of fluorescence at low temperature by ferricyanide are available.³⁹ However, these experiments indicated a series of other experiments which have provided further evidence that P_{680}^+ quenches fluorescence.

(38) R. Lozier and W. L. Butler, unpublished results.

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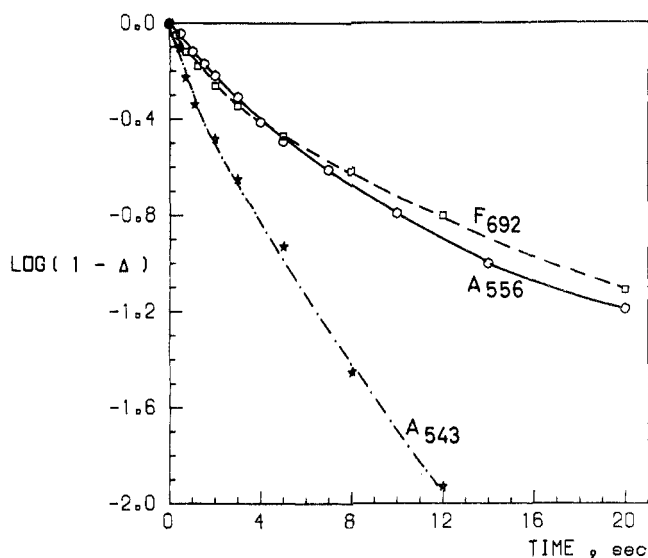


Figure 4. Semilogarithmic plots of the time course of the relative changes of absorbance at 543 and 556 nm and of fluorescence at 692 nm after the onset of irradiation. Δ is the fraction of the maximal change. Chloroplasts in 55% glycerol (1 mg of chlorophyll/ml in a 1-mm cuvet) were irradiated with 630-nm actinic light ($165 \mu\text{W}/\text{cm}^2$) at -196° .

Figure 4 shows semilogarithmic plots of the time course curves for the reduction of C-550 and the oxidation of cytochrome b_{559} , and the fluorescence yield in chloroplasts after the onset of irradiation at -196° .⁴⁰ It is clear that the photoreduction of C-550 proceeds more rapidly than the photooxidation of cytochrome b_{559} and that the fluorescence yield change has kinetics similar to those for the oxidation of cytochrome b_{559} . On the basis of the D·P·A model for the reaction center, the oxidation of cytochrome b_{559} may be taken to indicate the dark reduction of P_{680}^+ . Thus, the kinetics of the photoreactions at -196° indicate that the increase of fluorescence yield does not follow the photoreduction of the primary electron acceptor but rather the dark reduction of the photooxidized primary donor. Of the possible redox states of the primary electron transfer couple, $\text{P}\cdot\text{A}$, $\text{P}^+\cdot\text{A}$, $\text{P}^+\cdot\text{A}^-$, and $\text{P}\cdot\text{A}^-$, only $\text{P}\cdot\text{A}^-$ appears to result in a high fluorescence yield.

The temperature dependence of each of the three photoreactions was examined at temperatures between -196 and -100° at an actinic light intensity that was well below any saturation effects.⁴⁰ The photoreduction of C-550 showed very little temperature dependence over this range. The photooxidation of cytochrome b_{559} was independent of temperature from -196 to -160° , but the extent of the reaction decreased at temperatures above -160° until at -100° no photooxidation of cytochrome b_{559} was observed. To the extent, however, that the reaction occurred at temperatures between -160 and -100° , the rate of the reaction was largely independent of temperature. These data indicate that at temperatures above -160° a secondary electron donor other than cytochrome b_{559} starts to function, and that by -100° the unknown secondary donor completely replaces cytochrome b_{559} .

Even though the rate of photoreduction of C-550

and the rate of photooxidation of cytochrome b_{559} showed little temperature dependence, the rate of the light-induced fluorescence yield increase was markedly dependent on temperature, being three to four times more rapid at -100° than at -196° .⁴⁰

The temperature dependence of fluorescence induction was reported previously from four different laboratories,⁴¹⁻⁴⁴ and each gave a different explanation based on the assumption that fluorescence yield was determined solely by the electron acceptor side of PSII. The phenomenon remained anomalous, however, and *ad hoc* assumptions of questionable validity had to be made to suggest why the primary photochemical electron-transfer reaction might have such a temperature coefficient^{41,44} or why the fluorescence yield at -196° might be influenced by secondary electron acceptors.^{42,43} The realization that fluorescence yield may also be determined by the oxidizing side of PSII⁴⁰ provides a reasonable explanation for the phenomenon: *i.e.*, that temperature dependence of the fluorescence induction is due to the temperature dependence for the dark reduction of P_{680}^+ . This temperature dependence is not the usual type due to the activation energy of a particular reaction; rather it appears to involve a switch, as the temperature increases from -160 to -100° , from one secondary donor, cytochrome b_{559} , to another unknown donor which transfers electrons to P_{680}^+ more rapidly.

The proposed influence of P_{680}^+ on fluorescence yield also provides an explanation for the time required for the fluorescence yield to increase after a flash. Mauzerall⁴⁵ showed that the fluorescence yield of *Chlorella* cells at room temperature reached a maximum about 20 μsec after a brief (10 nsec) saturating flash. Photoreduction of the primary electron acceptor would be expected to occur much more rapidly, probably within the lifetime of the excited reaction center chlorophyll. It was suggested⁴⁶ that the increase of fluorescence yield followed the dark reduction of P_{680}^+ and that the 20- μsec period represented the time for this reaction to reach completion. (The smaller fluorescence increase which Mauzerall found during the first 100 nsec was not considered.) The proposed reaction time (the order of 10 μsec) for the reduction of P_{680}^+ would appear to be inconsistent with the measurements of Döring, *et al.*,²⁸ which showed that P_{680}^+ (chl a_{11} in their terminology) decayed with a half-time of 200 μsec . It has been pointed out, however, that the absorbance changes measured by Döring, *et al.*, indicated only one P_{680} per 10^4 chlorophyll molecules,⁸ and it was suggested⁴⁶ that these measurements showed only a minor fraction of the P_{680} because most of the P_{680}^+ decays too rapidly to be observed in their measurements.

Under most circumstances the fluorescence yield of green plant systems appears to be determined solely by the redox state of the primary electron ac-

(41) B. Kok, *Nat. Acad. Sci.-Nat. Res. Council, Publ.*, No. 1145, 45 (1963).

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(44) S. Malkin and G. Michaeli in ref 24, p 149.

(45) D. Mauzerall, *Proc. Nat. Acad. Sci. U. S.*, **69**, 1358 (1972).

(46) W. L. Butler, *Proc. Nat. Acad. Sci. U. S.*, **69**, 3420 (1972).

(40) W. L. Butler, J. W. M. Visser, and H. L. Simons, *Biochim. Biophys. Acta*, **242**, 140 (1973).

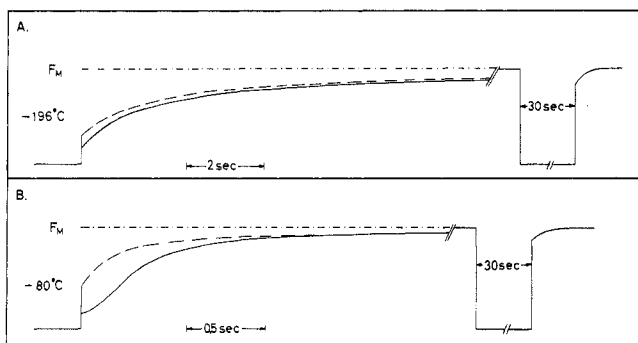


Figure 5. Time course curves for the light-induced fluorescence yield increase of dark-adapted chloroplasts before (solid curves) and after (dashed curves) a single saturating xenon flash. A, at -196° ; B, at -80° . The dot-dash line indicates the maximal F_M level. Note the time base in part B is four times faster than that in part A. Some decay of the fluorescence yield (and oxidation of C-550 not shown) occurs during a 30 sec dark period given after the F_M level was reached.

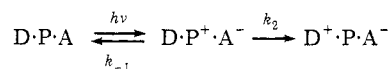
ceptor because the oxidized donor, P_{680}^+ , is rapidly reduced and does not reach sufficient concentrations to exert an influence on fluorescence. However, the influence of P_{680}^+ can be observed under special conditions such as at low temperature or at very high time resolution, and under these circumstances fluorescence yield does not follow the redox state of the primary acceptor. Important additional information to confirm or expand our understanding of the primary photochemistry of the PSII reaction centers would be provided if direct absorbance measurements of P_{680} were made and compared against the measurements of C-550, cytochrome b_{559} , and fluorescence yield.

The Back Reaction in the Primary Electron-Transfer Couple

The delay in the increase of fluorescence yield following a saturating flash was also observed at low temperature.⁴⁶ However, the magnitude of the fluorescence yield change resulting from a saturating flash at low temperature was a small fraction of the fluorescence yield change induced by continuous irradiation and was considerably less than the fluorescence yield increase which occurred after the same flash at room temperature. The relative ineffectiveness of a flash at low temperature was also confirmed by measuring fluorescence induction curves before and after a flash⁴⁷ (see Figure 5). After a single saturating flash at -196° the fluorescence yield was at a level somewhat above the F_0 level (Figure 5A, dashed curve) and the extent of the fluorescence induction increase was decreased approximately 15%; at -80° the fluorescence induction increase was decreased about 33% by a prior flash. The small increase of fluorescence yield induced by a flash at low temperature was consistent with the amount of C-550 reduced by the flash: approximately 15% of the C-550 was in the reduced state after a single saturating flash at -196° and approximately 33% after a flash at -80° .⁴⁷ Even though the energy of these flashes, from either a xenon lamp (16- μ sec duration) or a Q-switched ruby laser (20-nsec duration), was many times greater than that needed to

excite all of the reaction centers, the flashes were relatively ineffective when delivered at low temperature.

From a consideration of the photochemical system



it is apparent that the dark reaction, k_2 , is needed to stabilize the charge separation in the PSII reaction centers. At room temperature, k_2 must be faster than the back reaction, k_{-1} , in order for the stable photochemical products, D^+ and A^- , to be formed with good yield. As the temperature is lowered, however, k_2 apparently slows down relative to k_{-1} so that the back reaction becomes more important. Thus, following a single saturating flash at low temperature most of the reduced C-550 back reacts with the oxidized P_{680} .

The measurements of Floyd, *et al.*,⁴⁸ on P_{680} and cytochrome b_{559} changes induced by a short laser flash at -196° must have been primarily measurements of the back reaction. They interpreted their measurements to indicate that the photooxidized P_{680} was reduced in the dark by cytochrome b_{559} and that this dark reaction had a half-time of about 4.5 msec at -196° . However, the magnitude of their absorbance changes indicated that much more P_{680} recovered after the flash than could be accounted for by the amount of cytochrome b_{559} oxidized. Their results are consistent with P_{680}^+ being reduced primarily by the back reaction with only a small amount being reduced by cytochrome b_{559} . Their measurements would thus indicate the kinetics of the back reaction at -196° ; the kinetics for the oxidation of cytochrome b_{559} would, of necessity, follow the decay of P_{680}^+ and would appear to have the same half-time. In actuality, however, the rate constant for the oxidation of cytochrome b_{559} by P_{680}^+ at -196° is probably seven to ten times slower than the rate constant for the back reaction between P_{680}^+ and C-550 $^-$.

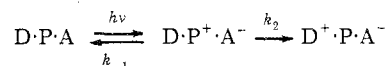
The loss of photochemical efficiency due to the back reaction should result in lower quantum yields for the photoreduction of C-550 in continuous light. At temperatures above -100° the fluorescence yield increase closely follows the photoreduction of C-550, so that fluorescence yield can be used as an index of C-550 reduction. The rate of fluorescence yield increase measured at room temperature in the presence of DCMU was found to be three times faster than the rate of increase at -100° ,⁴⁷ thereby confirming that the quantum yield of the primary photochemistry is less than optimal even at -100° . Thus, the temperature dependence of the reaction, k_2 , imposes a temperature dependence on the fluorescence induction by two mechanisms: (1) as k_2 slows down relative to k_{-1} more losses occur in the back reaction; and (2) at temperatures below -100° where cytochrome b_{559} begins to function as the electron donor, the quencher, P_{680}^+ , begins to accumulate as a transient and the fluorescence yield follows the dark reduction of P_{680}^+ .

(47) W. L. Butler, J. W. M. Visser, and H. L. Simons, unpublished results.

(48) R. A. Floyd, B. Chance, and D. Devault, *Biochim. Biophys. Acta*, 226, 103 (1971).

Intensity Dependence of PSII Reactions

The simple D·P·A model



accounts for most of the photochemical activities attributable to the PSII reaction centers. However, the light intensity dependence for these reactions is not readily accommodated by this model. The simple model of independent reaction centers would predict that the rate of photoreduction of C-550 should be proportional to light intensity and that the photooxidation of cytochrome b_{559} should saturate at a maximal rate determined by k_2 . Measurements of the quasi rate constants (reciprocal half-times) for the photoreduction of C-550 and the photooxidation of cytochrome b_{559} at -196° ⁴⁰ showed that the rates of both reactions were linear with light intensity at low intensities, below $200 \mu\text{W}/\text{cm}^2$, but fell off in a parallel fashion at higher intensities. The falling-off of the rate of C-550 photoreduction at the higher light intensities suggests a decrease in the apparent quantum yield of that reaction. Such a decrease in the quantum yield would occur if the back reaction, k_{-1} , were stimulated by light. It is possible that P_{680}^+ absorbs light (P_{680}^+ is postulated to quench fluorescence) and that the back reaction is facilitated when P_{680}^+ is excited. Such a photostimulation of the back reaction should be favored at higher light intensities because of the greater accumulation of P_{680}^+ , but there is no independent evidence for such

a mechanism. Siggel, *et al.*,²⁹ presented evidence that one DCMU molecule inactivated two PSII reaction centers. If these D·P·A reaction centers actually function in pairs, the back reaction might be more probable at the higher light intensities which would favor higher degrees of charge accumulation in the reaction center pairs. While such speculations should probably be restrained, to prevent their overproliferation, the anomalous intensity dependence of the photoreaction at low temperatures holds the promise that further investigations of the kinetics of the primary PSII reactions may provide new insights into the nature of the PSII reaction centers and the primary photochemical reactions.

C-550 vs. Q

The absorbance measurements of C-550 provide a more direct indication of the primary electron acceptor of PSII than the fluorescence yield measurements attributed to Q. Since fluorescence yield appears to be affected by both the primary electron acceptor and the primary donor, the use of the symbol Q, which represents quencher, for the primary electron acceptor could be ambiguous in certain cases. It seems preferable to replace Q with C-550 as indicated in Figure 1 and to relate the fluorescence yield changes attributable to the acceptor to changes of C-550. However, C-550 is an operational term dependent on a specific absorbance change, and in many cases where that absorbance change is not being measured or discussed a more generic symbol such as A (or A_{II}) might be more appropriate to use for the primary electron acceptor of PSII.

Systematic Approaches to the Chemical Synthesis of Polysaccharides

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Living organisms exist as structures with mechanical strength and some degree of extensibility, with the ability to grow, move, and reproduce, to localize foods and fluids, and to manipulate information. The organic structural elements that mediate these functions within living organisms for the most part belong to three classes of macromolecules—the proteins, the nucleic acids, and the polysaccharides.

The functions of proteins and nucleic acids are

widely recognized, but the roles played in living tissue by polysaccharides and oligosaccharidic side chains of other macromolecules are less appreciated. Polysaccharides are found as major portions of the exoskeletons of insects and arthropods and cell walls of plants and microbes. In many organisms they act as reserve foodstuffs and important components of intercellular, synovial, and ocular fluids, mucous secretions, blood serum, etc. They also constitute protective capsules of some of the most virulent microorganisms, capsules which, nevertheless, carry information which activate mammalian defenses: the immune, interferon, and properdin systems.

In a sulfated form some polysaccharides inhibit blood clotting. Oligosaccharidic side chains of glyco-

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