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Physical Mechanisms in Photosynthesis: Past Elucidations and Current Problems*

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Photosynthetic tissues are organized functionally into aggregates of light harvesting pigments (mainly chlorophylls, carotenoids, and phycobilins) associated with photochemical reaction centers (1, 2). The pigments absorb light and deliver the energy to the reaction centers, where an oxido-reductive photochemistry ensues. The primary photoproducts, oxidizing and reducing entities, serve as starting points for electron transport that is coupled to phosphorylation. In known cases, the primary photochemical electron donor, at the reaction center, is a chlorophyll (Chl) or bacteriochlorophyll (BChl). In the specialized context of the reaction center this donor is generally designated *P* (for pigment) followed by a number signifying the peak of the long wave absorption maximum: P700, P870, etc. (3, 4).

This organization defines certain physical problems:

(*a*) How is energy, absorbed by the light harvesting pigments, delivered to the reaction centers?

(*b*) What are the details of the photochemical process?

(*c*) How are the primary photoproducts used effectively and safely, without wasteful recombination or harmful indiscriminate reactions with the surroundings?

We shall consider these questions in turn, especially as related to the photosynthetic bacteria.

ENERGY TRANSFER AND FLUORESCENCE

Fluorescence in photochemical systems

The mechanism of energy transfer has been studied by measurement of the fluorescence emitted by the light harvesting Chl (or BChl) in relation to the chemistry occurring at the reaction centers. The intensity of fluorescence measures the concentration of singlet excited states, or excitation quanta, in the system. These excitations are the direct result of light absorption by the light harvesting pigments. In a steady state† the fluorescence also reflects the rate at which excitation is being quenched by non-fluorescent pathways. The more the "dark" quenching, the less the yield of fluorescence. The dark quenching encompasses dissipation into heat and utilization for photochemistry.

These relationships can be formulated as

$$\phi_f = k_f/(k_f + k_d + k_p) \qquad [1]$$

where ϕ_f is the quantum yield of fluorescence (quanta emitted/quanta absorbed) and the *k*'s are first-order rate constants for the processes by which singlet excitation quanta become lost: k_f for fluorescence, k_d for radiationless de-excitation (conversion into heat), and k_p for the event that leads eventually to photochemistry. It is usually assumed, for lack of information to the contrary, that k_f and k_d do not vary under changing physiological conditions, but the value of k_p may depend on the functional conditions of the reaction centers.

The migration of energy, and its trapping by photochemical reaction centers

Let us consider first those hypotheses by which a singlet excitation quantum in the light harvesting system is converted locally to some other (metastable) state, and the new state carries the energy to a reaction center. This new state might, for example, be a triplet excited state or an electron–hole pair in the ensemble of Chl molecules. It is not a source of the "prompt" (short lived) fluorescence that characterizes the singlet excited state. In such a model, if the reaction centers were altered so as to be unable to accept and process the energy, the most immediate result would be an increase in the population of metastable states. To a first approximation this would not affect k_p, which in this model is the rate constant for singlet → metastable conversion. The states of the reaction centers would have no effect, or at most a remote and indirect one, on the intensity of fluorescence.

Now suppose instead that the singlet excitation quanta are quenched at the reaction centers, by a process that depends on the state of the reaction center. Specifically, consider a specialized Chl or BChl, *P*, that can receive an excitation quantum from the light harvesting pigment and can then donate an electron so some acceptor, *A:*

$$P,A \xrightarrow{h\nu} P^*,A \rightarrow \rightarrow \rightarrow P^+,A^-$$
$$\uparrow \quad \text{(dark)} \quad \downarrow$$

where P^* denotes *P* in the singlet excited state. The "dark" restoration of the state "P,A" must be completed before the reaction center can perform its function again. Regarding the reaction center as a photochemical trap for singlet excitation quanta, the trap is "open" in the state P,A but becomes "closed" in any of the states P^+, A; P, A^-; or P^+, A^-.

When all the traps in a sample are open, k_p has its maximum value and the fluorescence is minimal. When all the traps are closed, $k_p = 0$ and ϕ_f has the maximum value of $k_f(k_f + k_d)$. In a typical experimental observation (5, 6) a sample is illuminated and the yield of fluorescence (from the light harvesting Chl or BChl) is seen to rise as traps become driven into a closed state.

This kind of hypothesis has been borne out by experiments

Abbreviations: Chl, chlorophyll; BChl, bacteriochlorophyll; *P*, pigment; Cyt, cytochrome; PMS, phenazine methosulfate.

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† A quasi-steady state is attained if the time constants for excitation and de-excitation (about 10 nsec) are short compared with the time scale of observation, and compared with the time constants for environmental (e.g., chemical) changes that affect the fluorescence.

with photosynthetic bacteria (5–7) and also with green plants in relation to the oxygen-evolving "photosystem II" (8–10).

The states of the reaction centers can be monitored independently, to a limited extent. In cells or subcellular (chromatophore) preparations of some photosynthetic bacteria such as *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum*, aerated and depleted of electron donating substrates, the conversion from P to P^+ can be seen as a light-induced bleaching near 870 nm. The fluorescence rises during illumination, in coordination with this bleaching. The quantitative relationship is as if at any instant, k_p is simply proportional to the fraction of P that has not yet become bleached (5–7). The simplicity of the relationship suggests that the model is correct and that under these conditions, the closing of traps is associated exclusively with the conversion from P to P^+; the state P,A^- does not play an important role. But under a reducing environment the flow of electrons to P^+, causing the reaction $P^+ + e^- \rightarrow P$, may be so rapid that no significant light-induced bleaching of P can be observed. Nevertheless the fluorescence from the light harvesting pigment may rise during illumination (6), suggesting that the traps are becoming closed on the "acceptor" side:

$$P,A \xrightarrow{h\nu} \rightarrow \rightarrow P^+,A^- \xrightarrow[\text{rapid}]{} P,A^-$$

This interpretation cannot be tested directly because of the difficulty of any direct observation of the conversion of A to A^-; we are just beginning to learn how to do this with purified reaction center preparations (see later).

In green plant tissues, most of the fluorescence, and all of the part that varies under physiological conditions, is associated with the oxygen-evolving system II. Although neither the primary photochemical electron donor nor the primary acceptor (usually symbolized Chl_{II} and Q, respectively) has been detected with certainty, an extensive phenomenology has been developed relating these hypothetical entities to fluorescence, oxygen evolution, and electron flow through the reaction center to some ultimate acceptor (Hill reaction) (8–12). Some conclusions that seem appropriate at present are:

(*a*) The yield of fluorescence does change in a way that reflects the states of the traps or reaction centers.

(*b*) The flow of electrons from water (oxygen evolving chemistry) to the "system II" trap is normally so rapid that the trap remains open on the oxidizing side; accumulation of oxidized Chl_{II} is negligible. Closure of the trap, and consequent high fluorescence, is associated with the reduction of Q.

(*c*) Oxidants close to the reaction center, possibly including oxidized Chl_{II}, may accumulate when the flow of electrons from water is impaired: by heating, by washing chloroplasts with Tris buffer, by ultraviolet irradiation, or through manganese deficiency. Electron flow to the reaction center can then be restored by addition of an artificial donor such as hydroquinone.

(*d*) A rapid cycling of electrons from reduced Q to oxidized entities near (and perhaps including) Chl_{II} is possible.

(*e*) Although high fluorescence is associated with the reduction of an entity "Q" (for quencher), that entity is not established conclusively to be the primary electron acceptor. Also there may be two kinds of "Q" with different oxidation–reduction properties.

With respect to green plant system I, for which P700 has been implicated as a primary electron donor, no component of the fluorescence shows variations that can be related sensibly to the chemical state of this system. We shall return to this problem later.

Light absorption, lifetime of the excited state, and yield of fluorescence

There is a useful pair of relationships between the intensity of absorption, the lifetime of the excited state (measured as fluorescence lifetime) and the quantum yield of fluorescence of a pigment. Imagine for the moment that the radiative pathway, fluorescence, is the only mechanism for de-excitation available to an excited molecule. The excited state will then have a mean lifetime τ_0, called the intrinsic lifetime, before it decays by fluorescence. This intrinsic lifetime is governed by the probability (per unit time) of fluorescence; indeed it is the inverse of that probability. On the other hand, the probabilities for absorption and fluorescence, being governed by the same physical considerations, are proportional to each other. Therefore, the probability of absorption is proportional to the inverse of the intrinsic lifetime of the excited state. Absorption probability is measured by the area under the appropriate absorption band; thus

$$1/\tau_0 = \text{Constant} \times (\text{absorption-band area}); \qquad [2]$$

see ref. 13 for the details of this formulation.

Eq. **2** allows a simple computation of τ_0, the lifetime that would prevail if the quantum yield of fluorescence were 100%. But other processes, exemplified by k_d and k_p in Eq. **1**, compete with fluorescence for quenching of the excited state. These other processes shorten the mean lifetime, and reduce the fluorescence yield in the same proportion. Thus, if the yield of fluorescence is ϕ_f and the "actual" mean lifetime of the excited state is τ, we can write

$$\tau = \phi_f \tau_0. \qquad [3]$$

The value of τ_0 computed from the absorption band area is 15 nsec for Chl *a* and 20 nsec for BChl. For Chl *a* in ether, the lifetime is 5 nsec and the fluorescence yield is 33% (14), in exact agreement with Eq. **3**. In green plants, the lifetime and yield are about 0.5 nsec and 3%, respectively (15), again in good agreement. In several types of photosynthetic bacteria the yields (16) and lifetimes (17, 18) of BChl fluorescence range from about 2–10% and 0.4–2 nsec, respectively, again in harmony with Eq. **3**.

Summary and anomalies

In summary, there is abundant simple evidence that energy absorbed by light harvesting pigments reaches the reaction centers in the form of singlet excitation quanta, and no compelling evidence to the contrary. This conclusion can be made for green plant system II and for certain photosynthetic bacteria, especially *Rps. spheroides* and *R. rubrum*. In these systems, with photochemical traps fully functioning, the mean lifetime of singlet excitation is usually about 0.5 nsec. This appears to be the time needed for migration of the energy to the traps.

For green plant system I there are several possibilities to account for the absence of any fluorescence that varies with the state of P700:

(*a*) P700 is not the major sink for excitation quanta. This is

belied, at least under some conditions, by the high efficiency of light-induced oxidation of P700 (19).

(*b*) The energy is transferred to the reaction centers, and quenched there, so efficiently that the yield of fluorescence is undetectably small.

(*c*) The singlet excited state is transmuted by an unknown process independent of the state of the P700. This process might even involve a "true", not yet detected, primary photochemical electron donor that can in turn oxidize P700. In this view, P700 could be a safety device to get rid of excess oxidants when these accumulate.

Because we cannot choose among these alternatives, we cannot pretend to a satisfactory understanding of primary energy transduction in green plant system I. But in the photosynthetic bacteria we can accept, for the present, the simple view that a singlet excitation quantum migrates to a reaction center where it generates an electrically polarized state, precursor of the more stable couple P^+,A^-.

We conclude this part of the discussion by listing three anomalies.

First, evidence exists (20, 21) that at least in *R. rubrum*, there is more than one kind of photochemical system, each with its characteristic reaction center. Whether the variations in fluorescence are related to the conditions at more than one kind of reaction center remains to be settled.

Second, in at least one photosynthetic bacterium, *Ectothiorhodospira Shavoshnikovii*, the component of fluorescence that varies with the states of the reaction centers has a lifetime far less than 0.5 nsec (45). When the traps are all open, the lifetime may be as short as 5×10^{-11} sec, suggesting very rapid migration of excitation quanta to the traps. Perhaps this is the case for green plant system I as well.

Third, we now recognize that the yield of fluorescence associated with green plant system II varies with the electrochemical state of the membrane (thylakoid membrane) that carries the photosynthetic apparatus. Specifically, a quenching of the fluorescence is correlated with the development of a gradient of H^+ concentration across the membrane (22, 23). This may happen in photosynthetic bacteria as well (Sherman, unpublished observations). The mechanism is unknown.

PHOTOCHEMISTRY; CONSERVATION AND UTILIZATION OF THE PHOTOPRODUCTS

Photochemical reaction centers from photosynthetic bacteria

Preparations of photochemical reaction centers made from carotenoidless mutant *Rps. spheroides* (24, 25) are composed of a hydrophobic protein, of molecular weight probably between 60,000 and 140,000, to which BChl and bacteriopheophytin are bound through hydrophobic interactions. There are probably two bacteriopheophytin and three or four BChl molecules attached to each protein molecule (26). The BChl is responsible for absorption bands near 800 and 865 nm; the 865-nm band is identified as P870 in the intact cell. The P870 is bleached reversibly by light; this is identified as a photochemical oxidation of the pigment. No other prosthetic groups, with the possible exception of an iron atom (25), have been found in well purified reaction centers. The identity of the primary electron acceptor remains a mystery; perhaps it is nothing but a locus of electron affinity generated by a special configuration in the protein.

The reaction center protein can be dissociated with sodium dodecyl sulfate into three distinct subunits (27) whose apparent molecular weights based on electrophoresis in polyacrylamide are 19,000, 23,000, and 27,000. If these weights are correct, and if the amount of protein is proportional to the amount of Coomassie Blue stain that it binds in the acrylamide gel, the ratio of the subunits is 1:1:1. This would give a molecular weight of 69,000 for the smallest assemblage of subunits.

Serological experiments (W. R. Sistrom, R. K. Clayton, and R. L. Berzborn, unpublished data) and analysis by acrylamide gel electrophoresis (27) show that this protein makes up about one fifth of the total protein of chromatophores prepared from wildtype *Rps. spheroides*. From such chromatophores this singular protein can be isolated in fairly pure form, but accompanied by the light harvesting pigments, by following the same procedure that yields a reaction center preparation when applied to the carotenoidless mutant strains. The crux of the procedure is to treat the chromatophores with the detergent lauryl dimethyl amine oxide, centrifuge at about $200,000 \times g$ for 2 hr, and discard the pellet. The light harvesting pigments may be bound to the reaction center protein, but they are certainly bound to other proteins in the chromatophore as well.

Sistrom's nonphotosynthetic mutant strain PM-8 of *Rps. spheroides* lacks the reaction center protein. We have shown this both serologically and through analysis by acrylamide gel electrophoresis (27). The light harvesting BChl and carotenoids are bound to other proteins in PM-8; the photochemically active P870 is missing.

The P870 in reaction centers can be oxidized by illumination:

$$P,A \xrightarrow{h\nu} P^+,A^-$$

and the electron can be passed on from A^- to a secondary acceptor such as added ubiquinone or ferricyanide:

$$P^+,A^- + B \rightarrow P^+,A + B^-$$

where B denotes the secondary acceptor. The light-induced state P^+,A may be stable for several seconds, allowing some leisure in attempts to analyze the material while the P870 is in either its reduced or its oxidized form. The chromophores can be extracted from the reaction centers by dilution of the sample with methanol and centrifugation away of the denatured protein. When this is done, the reduced P870 appears as BChl, with an absorption maximum at 770 nm, in the methanolic solution. But extraction of the oxidized form, P^+, yields a bleached form of BChl, presumably $BChl^+$. The bleached form in the methanolic solution can be restored to the unbleached form by addition of ascorbate (S. C. Straley, unpublished data), but the regenerated absorption band is at 780 nm rather than 770 nm. These experiments show that one can prepare a natural photoproduct, oxidized P870, and then study it *in vitro*.

Fluorescence of reaction centers in relation to their chemical activities

The photoproducts in reaction centers can be made to react with external electron donors and acceptors; the possibilities are summarized in Fig. 1.

If the surroundings contain a good donor of electrons such as reduced cytochrome *c* (Cyt *c*), the conversion from P^+ to P can be so rapid that the accumulation of neither P^+,A nor P^+,A^- is significant during illumination. The population

of reaction centers then shifts from the state P,A to P,A^- during illumination. Alternatively, if a good acceptor (ubiquinone or ferricyanide) is present, the principal states are P,A and P^+,A.

These transformations are attended by variations in the yield of fluorescence emitted by P870 (26, 28, 29) (Note that in these reaction-center preparations, there is no light harvesting BChl to emit fluorescence. Fluorescence from P800 is negligible, and that from BPh can be avoided by exciting at wavelengths greater than 800 nm).

The states P^+,A and P^+,A^- are nonfluorescent because the emitting species, P, is missing. During illumination of reaction centers without added electron donor (upper pathway in Fig. 1) the fluorescence band of P870, centered near 900 nm, disappears along with the absorption band centered near 865 nm. This is shown in Fig. 2, curve *a*. On the other hand, if an electron donor such as reduced Cyt *c* or phenazine methosulfate (PMS) is present, the fluorescence rises during illumination, as in curve *b*. The most direct interpretation of this rise is that the state P,A^- is being formed (lower pathway in Fig. 1). In this state the P870 is more strongly fluorescent because the excited state P^*,A^- cannot be discharged photochemically.

The problem of the photochemical electron acceptor

Following the interpretation of the foregoing paragraph, the fluorescence reveals properties of the primary electron acceptor. The initial fluorescence, f_0 in Fig. 2, can be driven to values approaching f_{max} by chemical reduction, presumably because A is being reduced to A^-. A titration of this effect (29) shows that the system A/A^- has a mid-point potential of -0.05 V, independent of the pH, with the stable oxidized and reduced forms differing by one electron.

The indication that the acceptor can hold just one electron is confirmed by experiments (26) showing that in the absence of secondary electron acceptors, but with an excess of reduced Cyt *c* present, just one equivalent of Cyt *c* can be oxidized photochemically for every equivalent of P870:

$$P,A + \text{Cyt} \xrightarrow{h\nu} P^+,A^- + \text{Cyt} \rightarrow P,A^- + \text{Cyt}^+$$

This stoichiometry can be altered by addition of ubiquinone as a secondary electron acceptor; then two additional equivalents of Cyt can be oxidized for every mole of ubiquinone added.

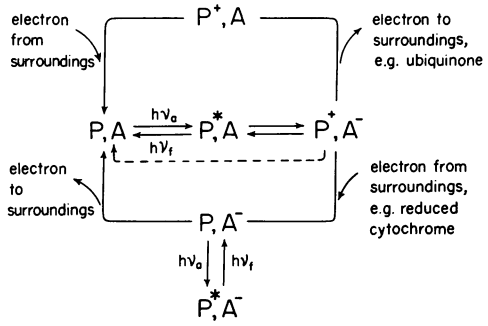

FIG. 1. A diagram of a model for photochemical oxido-reduction and subsequent electron transport in photochemical reaction centers from *Rhodopseudomonas spheroides*. P denotes P870, P^* is P in the lowest singlet excited state, and P^+ is oxidized P. The hypothetical electron acceptor in the photochemical act is designated A. The surroundings are meant to include endogenous as well as external electron donors and acceptors. Steps involving light absorption and fluorescence are marked $h\nu_a$ and $h\nu_f$.

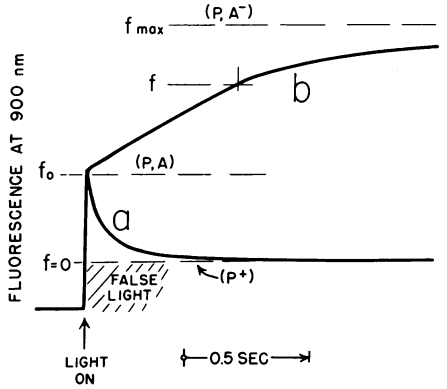

FIG. 2. Time course of the fluorescence at 900 nm emitted by reaction centers from *Rps. spheroides* during constant illumination. Reaction centers, 1.0 μM, in 0.01 M Tris·HCl buffer (pH 7.5) with 0.1% lauryl dimethyl amine oxide and 5 μM ubiquinone. Exciting light 800 nm; 4.0 mW/cm^2. *Curve a:* No electron donor added. The fluorescence declined as the emitter, P870, became oxidized (and bleached) to P^+. The "false light" signal with all P870 bleached is ascribed to scattered exciting light and emission from chromophores other than P870. *Curve b:* With 25 μM bovine cytochrome *c*, added in the reduced form. The P870 was kept predominantly reduced by the cytochrome during illumination, and the fluorescence rose (ultimately to f_{max}) as the photochemistry proceeded. The rise is attributed to reduction of the primary electron acceptor A. The maximum level, associated with "P,A^-", could also be obtained by adding $Na_2S_2O_4$.

The same conclusions have been reached (26) by analysis of the shapes of "fluorescence rise" curves such as curve *b* in Fig. 2. Such analysis can give information as to the number of quanta needed to drive a sample of reaction centers from the state P,A to P,A^-. In the absence of secondary electron acceptors, about one or perhaps 1.5 quanta are needed for every P870. Independent measurements (26, 30) show that 1–1.5 quanta suffice for the oxidation of one P870 (and by implication, the reduction of one A to A^-). The exact computed value depends on an assumption as to the optical absorption coefficient of P870; this remains somewhat uncertain (26).

There are two other pieces of information that might help to identify the primary acceptor. First, a very broad (several thousand gauss bandwidth) light-induced electron-spin resonance signal has been detected in reaction centers from *Rps. spheroides*, (25) in addition to the familiar narrow one that signals the presence of P^+. The broad signal could be a manifestation of A^-.

Second, we have detected light-induced optical absorption changes (ref. 31, and Straley and Clayton, unpublished data) that might be identified with the interconversion of A and A^-. These absorption changes are perceived when reaction centers are illuminated in the presence of an electron donor such as PMS so as to suppress the appearance of oxidized P870:

$$P,A + \text{PMS} \rightarrow P,A^- + \text{PMS}_{ox}$$

After any changes attributable to the oxidation of PMS have been discounted, we see a residual set of absorption changes that could reflect the conversion of A to A^-. Another approach is to illuminate reaction centers in the presence of mixed

ferri- and ferrocyanide. One can detect a slow absorption change after the rapid initial "light on" reaction, and the details of this process suggest that the slow change signifies the reaction

$$P^+,A^- + Fe^{+++} \rightarrow P^+,A + Fe^{++}$$

When the reduction of ferricyanide (Fe^{+++}) to ferrocyanide (Fe^{++}) has been discounted, the net optical change reflects "$A^- \rightarrow A$".

Both kinds of measurement show that the conversion from A to A^- (in our hypothesis) is attended by the following absorption changes: An absorption band appears, centered at 455 nm. There are bathychromic shifts ("red-shifts") of bands near 300, 530, and 760 nm.

The bands near 530 and 760 nm can be identified with bacteriopheophytin, and the shifts of these bands might be due to local electric fields acting on the bacteriopheophytin molecules. Such fields could arise as a result of the photochemical separation of electric charge in the reaction centers. The 800- and 865-nm bands sometimes show small blue-shifts that accompany the red-shifts near 530 and 760 nm; these shifts are distinct from the much larger changes (blue-shift at 800 nm and bleaching at 865 nm) that signal the oxidation of P870. All of these effects may become useful in sensing and mapping the movements of electrons in the reaction centers. At present they simply illustrate the need for caution in interpreting the absorption changes associated with the reaction "$A \rightarrow A^-$".

Our ignorance concerning the primary photochemical electron acceptor in reaction center preparations from *Rps. spheroides* is representative of our lack of knowledge about this entity in general. Mid-point potentials have been estimated from the way that the redox potential affects the fluorescence of light harvesting Chl or BChl, by use of the assumption that k_p (Eq. **1**) becomes zero when the acceptor becomes reduced. Such experiments suggest that in several types of photosynthetic bacteria (32), the acceptor is a "one-electron" agent with a mid-point potential in the range -0.05 to -0.16 V, independent of the pH. Our results with reaction centers are consistent with these measurements for *Rps. spheroides*. Similar conclusions have been drawn (33, 34) from the ability of reaction center or chromatophore preparations to mediate the photochemical reduction of added substances of various mid-point potentials (methylene blue, indigo sulfonate dyes, etc.).

Chloroplasts and other preparations from green plants are able to effect the photochemical reduction of viologen dyes and other difficult-to-reduce substances, to extents that signify a reducing potential of about -0.6 V for photosystem I (35). In this ability to form very strong reductants photochemically, green plant system I differs strikingly from any of the bacterial systems thus far characterized.

The fluorescence of Chl associated with green plant photosystem II varies with redox potential in a way that suggests two acceptors, or "quenchers", of mid-point potentials about -0.03 and -0.3 V at pH 7 (36). These show a pH dependence of 60 mV per pH unit and a "one-electron" redox titration curve.

We do not know what any of these presumed acceptors are.

Knowledge and speculation about the photochemical act

Returning to the behavior of reaction centers prepared from *Rps. spheroides*, the quantum yield of fluorescence of "P,A" if 4×10^{-4}. Therefore, from Eqs. **2** and **3**, the lifetime of the excited state P^*,A is 8 psec (28). The most likely fate of this excited state is one that leads to P^+,A^- since the quantum efficiency for the photochemistry is 70% or greater. We can conclude that the first step in the conversion of singlet excitation energy to chemical potential is an event with a half-time of about 8 psec. This event could be the displacement of an electron from P toward A, forming a charge-transfer state that settles into a more stable configuration (P^+,A^-) through nuclear rearrangements. We have no basis for more detailed speculation at present.

Secondary oxidations and reductions; conservation of energy and prevention of undesirable reactions

The earliest electron transfer events following the primary photochemical process have been delineated in photosynthetic bacteria, especially by Parson (37, 38) using laser flash excitation and measurement with time resolution better than 1 μsec. In extracts of *Chromatium*, the primary formation of "P^+,A^-" is followed, with a time constant of about 1 μsec at room temperature, by the oxidation of one or another Cyt of the "*c*" type, C553 or C555. Then, with a characteristic time of about 50 μsec, the reaction center returns to the state P,A (in Parson's terminology, P,X) by discharging the electron from A^- to a secondary acceptor. The principal secondary acceptor is probably ubiquinone; this substance abounds in the pigmented membranes of all photosynthetic bacteria and is carried over into some kinds of reaction center preparations (39).

The foregoing time constants vary widely among different kinds of photosynthetic bacteria: the half-time for Cyt oxidation ranges from 0.3 to 10 μsec (40). In every case these secondary electron transfer events are rapid enough to consolidate the primary separation of oxidizing and reducing entities. The most direct return of electrons from A^- to P^+, involving no other recognized electron carriers, has a half time of 20–30 msec at temperatures from 1.3 to about 200°K, in chromatophore preparations and in reaction centers (41–43). At room temperature, this recombination appears to have a half-time of about 60 msec (43). In contrast, the transfer of electrons from Cyt to P^+ and from A^- to ubiquinone requires far less than 1 msec at room temperature in cells and chromatophores of photosynthetic bacteria. Thus, the secondary, energy-conserving electron transfers compete easily against any wasteful direct return of electrons from A^- to P^+.

The secondary electron transfers that occur in living cells can be mimicked in reaction center preparations, by addition purified mammalian Cyt *c* and ubiquinone to these preparations. The Cyt *c* appears to be bound electrostatically, and can transfer electrons to P^+ with half-time about 25 μsec at room temperature (44). The ubiquinone is bound, apparently by hydrophobic interactions (43), and can accept electrons from A^- with half-time far less than 20 msec. The reaction centers can therefore be used to reconstruct certain activities of the living cell, and these models show decisively how the primary conversion of light quanta to chemical potential can be consolidated by subsequent oxidations and reductions.

In green plant system II the primary oxidizing entity, perhaps oxidized Chl, is neutralized quickly by electrons from the chemical system that mediates the evolution of oxygen from water. When this process is arrested, as by washing chloroplasts with Tris buffer, the supply of electrons from water can be replaced by electrons from artificial donors

such as hydroquinone. But if no such donor is added, the accumulation of strong oxidizing entities is soon made evident by oxidative damage to a variety of components of the system (12). Light harvesting Chl, carotenoid pigments, and presumably many other substances, such as Cyt, succumb to indiscriminate photochemical oxidation under these circumstances. This damage can be halted by addition of a donor of electrons to the oxidizing side of system II. These experiments show how, at least in one photosynthetic system, the rapidity of secondary electron transfer events not only conserves and channels the energy, but also prevents the primary photoproducts from reacting with their surroundings in an uncoordinated and damaging way.

SUMMARY

By examining selected systems and preparations from photosynthetic materials, we have obtained partial insights into the physical mechanisms of photosynthesis. Certain traditional problems have been laid to rest, at least in principle, but many details remain to be understood. Foremost among the questions now outstanding are:

(*a*) How does the biochemistry of oxygen evolution work?

(*b*) What are the components of a reaction center for green plant photosystem II, and what is the primary electron acceptor in each of the various photosynthetic systems?

(*c*) How many distinct kinds of photochemical system can be found among the photosynthetic bacteria? How closely does any of them resemble green plant system I?

(*d*) What are the detailed steps that intervene between the singlet excited state of Chl or BChl and the appearance of primary oxidizing and reducing entities?

(*e*) What is unique about Chl and BChl as sensitizers of photochemical oxido-reduction? Is the primacy of these pigments in photosynthesis merely an evolutionary happenstance?

NOTE ADDED IN PROOF

P. A. Loach (verbal communication) has discovered a new light-induced electron-spin resonance signal in subcellular preparations from photosynthetic bacteria after treatment to remove iron. The signal has $g = 2.005$ and 7 gauss bandwidth. Its response to electron donors and acceptors suggests that it is a property of the primary electron acceptor, partner to the oxidation of P870. M. Okamuro, J. McElroy, and G. Feher (verbal communication) confirm that this signal is exhibited by reaction center preparations treated to remove iron. The broad signal described earlier by Feher (25) could be due to an interaction between the primary electron acceptor and an iron atom.

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