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Picosecond studies of primary charge separation in bacterial photosynthesis

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With the aid of light and two coupled photosystems containing chlorophyll, green plants remove electrons from water, releasing O_2, and convey them to CO_2, reducing it to simple sugars and thence to carbohydrate. Photosynthetic bacteria operate more simply, employing a single photosystem. They reduce CO_2 but require substrates more easily oxidized than water, e.g. sulphide and succinate. Bacterial reaction centres free of the bulk chlorophyll that performs the light-harvesting function can be isolated. The reaction centre is the site where the electronic energy of the photoexcited molecule is converted to chemical potential. Thus bacterial reaction centres are ideal subjects for studying the details of this process by kinetic spectroscopy. Picosecond laser studies show that an electron is removed from the primary donor (a chlorophyll dimer) in 4 ps or less and transferred in several stages to the ubiquinone acceptor in *ca*. 200 ps. Remarkably, reverse electron transfer is several orders of magnitude slower. The paper discusses how Nature may have accomplished this.

1. Introduction

During the past 5 years, with the aid of new spectroscopic techniques based on ultra-short laser pulses, it has become possible to study the details of the primary charge separation process in bacterial photosynthesis (see Holten & Windsor (1978) for a recent review). Spectroscopic and kinetic studies with picosecond laser pulses have shown that electron transfer from the primary donor (a 'special pair' of chlorophyll molecules) to the ubiquinone acceptor takes place in about 200 ps. Even so, the transfer does not occur in a single step. Two or more stages are involved and the very first step is found to be exceedingly fast (less than 10 ps and perhaps as little as 4 ps). These times are several orders of magnitude shorter than had previously been assumed. Still more remarkable is the observation that the reverse electron transfer is three or more orders of magnitude slower, leading to a quantum efficiency very close to 100% for the primary charge separation. It is an intriguing challenge to seek to understand how Nature has accomplished this. Comparison of the new picosecond data on reaction centres of photosynthetic bacteria with the results of similar studies of electron transfer in model systems involving molecular solutions of photosynthetic pigments and various electron acceptors *in vitro* has been helpful in this regard. The purpose of this paper is to review the experimental data and discuss possible theoretical models. Some background on the primary events of photosynthesis will be given, with a brief account of the experimental techniques developed in our laboratory for picosecond spectroscopic studies.

2. Picosecond flash photolysis and spectroscopy

This is a direct extension to the picosecond region of the well known microsecond technique of flash photolysis with rare-gas filled flashlamps (Norrish & Porter 1949; Porter 1950). A mode-locked laser provides the picosecond excitation pulse. The spectroscopic flash for monitoring

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purposes is a picosecond continuum pulse obtained by focusing a portion of the laser pulse into a variety of optical media. A variable time delay (from a few picoseconds to several nanoseconds) between the two pulses is obtained by changing the difference in optical path length between the two beams. The apparatus developed by Magde & Windsor (1974) is shown in figure 1. A mode-locked Nd:glass laser, with single pulse selection (s.p.s.) and one stage of amplification, produces a 5–8 ps duration pulse of 15–20 mJ energy at 1060 nm. Single pulse selection is accomplished by using an optically triggered spark gap and Pockels cell. Passage of the 1060 nm pulse through a suitably orientated crystal of potassium deuterium phosphate gives second harmonic generation (s.h.g.) at 530 nm with a conversion efficiency of about 15%.

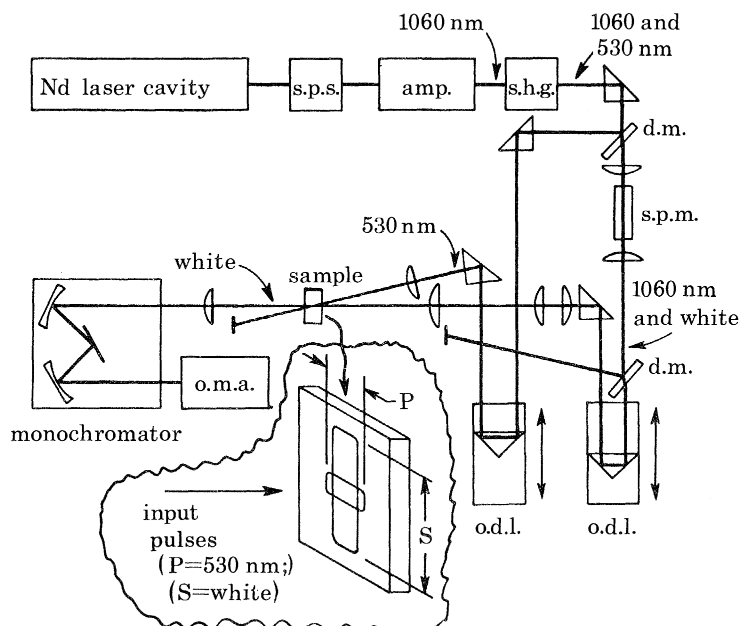

FIGURE 1. Diagram of apparatus for picosecond flash photolysis and spectroscopy.

The 530 and 1060 nm pulses are separated by a dichroic mirror that selectively reflects the 530 nm radiation while transmitting the 1060 nm pulse. After passage through an optical delay line (o.d.l.) consisting of a rooftop prism mounted on an optical rail, the 530 nm pulse (P for photolytic) impinges on the sample, usually a cuvette 1 or 2 mm thick. Any 530 nm light transmitted by the sample is intercepted by an optical stop. As a further precaution against scattered P light entering the spectrograph, it is arranged that the P beam makes a slight angle (10°) with the monitoring (S for spectroscopic) beam as in figure 1.

The key to the technique of picosecond flash photolysis is the picosecond continuum pulse. This is obtained by focusing the residual 1060 nm radiation into a cell 10 cm long containing carbon tetrachloride, CCl_4. Self-focusing and filamentation occur and a small fraction (*ca.* 0.1%) of the incident beam is converted into a broad continuum. The continuum retains both the short pulse duration and much of the spatial collimation of the pumping pulse. Thus, to all intents and purposes, we have a white-light laser. Another dichroic beam splitter separates the essentially undiminished 1060 nm pulse from the continuum. The 1060 nm pulse can be used for sample excitation or can be blocked, as in figure 1. The continuum (S) passes through a variable optical delay line and arrives at the sample at a predetermined time relative to the P

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pulse. By adjusting the two o.d.ls the time interval between S and P can be varied between zero and about 9 ns. Negative delays (i.e. S arrives before P) are also readily obtainable. These are useful in providing a record of the sample before excitation for control purposes and correspond to 'infinite delay' shots in conventional flash photolysis.

Optical density changes induced in the sample by the P pulse are measured in the following way. The inset at bottom centre of figure 1 shows the cross-sectional geometry of the two beams at the sample. The details of the geometry in this region are very important. The key point is that the probe beam is elongated along its vertical axis by means of a cylindrical lens so that it samples simultaneously the excited volume in the centre and unexcited *reference* volumes both above and below the excited region. The cross section of the excited volume can be adjusted in the range 1 mm to about 5 mm by means of a lens. The entire area is then imaged at about 1:3 diminution on to the slit of a spectrograph and, ultimately, on to either photographic film or a vidicon detector coupled to an optical multichannel analyser (o.m.a.). The absorption spectrum of a transient intermediate then appears sandwiched between two reference spectra of the unexcited sample, as in figure 2*a*. By having a reference spectrum both above and below a purported transient spectrum, we greatly reduce the risk of misinterpreting random fluctuations in the intensity of the continuum as genuine transient effects. For comparison we provide in figure 2*b* the photographic record of the decay of triplet 1,2-benzanthracene in solution (Porter & Windsor 1958) taken by conventional flash photolysis in the microsecond-millisecond region. Taken together, these two figures show how in two decades, aided by the laser, the time resolution of flash photolysis has been extended by six decades from 10^{-6} to 10^{-12} s.

The photographic method is almost essential for exploratory survey work on a previously uncharacterized system. It provides wide spectral coverage in a single shot, and the time history of whatever transient changes are present can be seen at a glance on a single film or plate by taking a short sequence of shots at different time delays. This is demonstrated in figure 2*a*, which shows picosecond flash photolysis results for octaethylporphinatotin(IV) dichloride ((OEP)$SnCl_2$)) (Magde *et al.* 1974*b*). The value of the wide spectral coverage provided by the photographic record is particularly evident here. The photograph clearly shows two regions of excited state absorption, a short-lived transient with a main absorption peak at *ca.* 450 nm and absorption extending to longer wavelengths, and a much longer-lived absorption in the blue with a peak at about 430 nm. Additional studies with the vidicon enable these two transient absorptions to be spectrally characterized and attributed respectively to the excited singlet S_1 state and the triplet T_1 state of the porphyrin. Broad-band picosecond spectra such as these are very helpful in choosing suitable wavelengths for subsequent quantitative kinetic measurements.

Although the photographic method is of great value for survey work, it is not the most convenient method for determining precise kinetic data. Therefore, once the photographic spectra reveal the best wavelengths to monitor, we replace the camera with a slit and use photoelectric detection, normally a Princeton Applied Research (PAR) 1205B vidicon tube coupled to a 500-channel optical multichannel analyzer (o.m.a.). We then monitor only a single narrow band of wavelengths, using the (o.m.a.) to obtain an intensity profile along the length of the slit. This is exactly equivalent to a densitometer trace taken vertically across the photographic spectrogram. The natural logarithm of the ratio of the measured intensity in the reference regions (averaged over the portions above and below) to that in the central excited region gives the absorbance change (ΔA) due to the transient absorption (or bleaching). Usually four or five

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shots are taken at a given wavelength and time delay, to obtain an average value and the standard deviation. Repeated measurements at the same wavelength and different time delays provide a plot from which the decay kinetics can be determined. By keeping the time delay constant and changing the wavelength, the difference spectrum at a given time delay can be obtained. In most cases, because of spectral overlap of the ground state and excited state

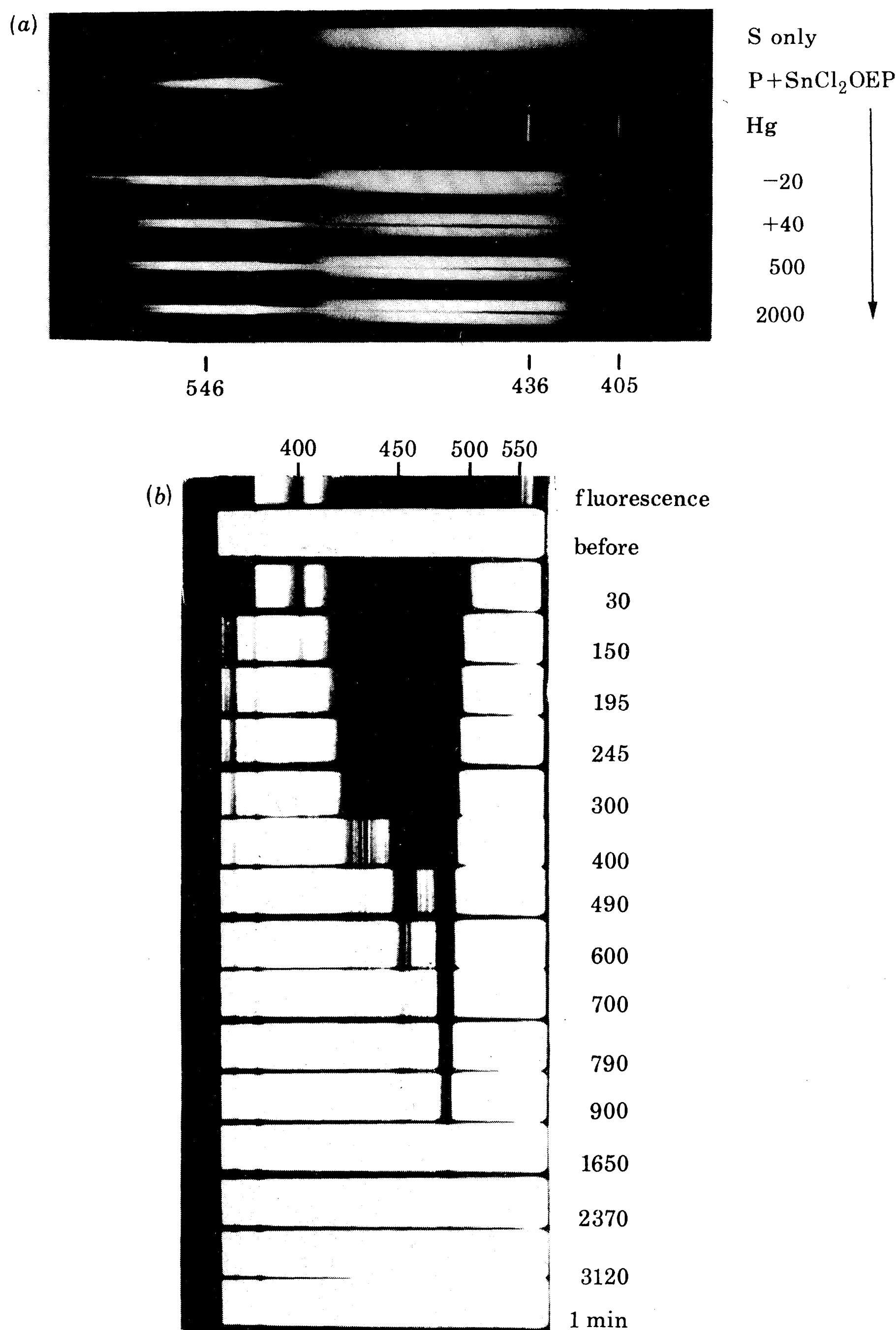

FIGURE 2. (*a*) Picosecond flash photolysis of tin dichlorooctaethyl porphyrin, showing decay of excited singlet absorption (short-lived) and triplet absorption (longer-lived). (*b*) Conventional flash photolysis of 1,2-benzanthracene in viscous paraffin solution showing decay of triplet absorption. Time scale in microseconds.

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absorption regions and because, at the laser powers used, significant depletion of the ground state is common, the spectrum obtained must be considered a 'difference spectrum' that represents the sum of the changes caused by ground state depletion and production of the excited state. This is especially true of photosynthetic systems in which spectral overlap of the various constituents pigments and associated molecules is common. By detailed studies it is usually possible to find wavelengths at which kinetic studies of one species can be made without interference by optical changes due to another species (see Rockley *et al.* 1975).

Very recently, we have interfaced our picosecond flash photolysis apparatus to a dedicated microprocessor (Holten *et al.* 1979*a*; Holten & Windsor 1980). This makes it possible to obtain picosecond transient spectra over a spectral range of 300 nm with a single laser shot. It also provides the advantage of computer processing of the experimental data. All of the experimental results described in this paper, however, were obtained by the more laborious point-by-point technique.

3. Background on photosynthesis†

In green plants, sunlight is absorbed by an antenna or light-harvesting system comprising many hundreds of chlorophyll molecules complexed to protein and situated in a lipid bilayer membrane. The excitation energy of electronically excited chlorophyll is rapidly conveyed by non-radiative mechanisms in times that range from 10 ps to about 1 ns, dependent upon species, to a special chlorophyll complex that acts as a trap. This is situated in a reaction centre (RC) complexed to other pigments and protein and to an electron acceptor, normally a quinone. Within the RC, charge separation takes place, the special chlorophyll complex being oxidized to a cation while the acceptor receives an electron and is converted to an anion. In this manner the energy of solar photons, transferred from the antenna to the RC as electronic molecular excitation, is captured within the RC as chemical potential. In green plants two coupled photosystems, called PS1 and PS2, cooperate to produce a strong oxidant capable, via an as yet poorly understood series of reactions, of oxidizing water to molecular oxygen and a strong reductant capable of reducing CO_2 to simple sugars and eventually carbohydrate. Described in another way, we can say that photosynthesis removes electrons from H_2O, thus releasing O_2, pumps them uphill in redox potential with the aid of two boosts from solar photons and finally places them on CO_2, thus allowing it to pick up hydrogen ions and be converted to a simple sugar. On a scale of redox potential this is illustrated in figure 3*a*.

Photosynthetic bacteria, like green plants, also contain an antenna system that harvests incident photons and transfers the excitation to an RC, but, unlike plants, bacteria employ only a single photosystem which more closely resembles PS1 in plants (figure 3*b*). The primary oxidant has a potential of about +0.45 V. This is insufficient to oxidize water, and bacteria require more easily oxidizable substrates, e.g. sulphide, succinate or thiosulphate. The primary reductant, as in plants, is coupled to the synthesis of ATP and the reduction of CO_2. A further advantage of photosynthetic bacteria is that, by suitable detergent treatments, RC preparations free of antenna pigments can be obtained (Clayton 1973). The presence of a single photosystem and the availability of isolated reaction centres have made bacterial RCs the preferred choice of most investigators for detailed studies of the process of primary charge separation. The majority of studies have been made on RCs of two species, *Rhodospeudomonas sphaeroides* and *Rhodospeudomonas viridis*, although other species such as *R. rubrum*, *C. vinosum* and *C. minutissimum* have been

† See Holten & Windsor (1978) for a more detailed account.

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used on occasion. Reaction centres of *R. sphaeroides* contain four molecules of bacteriochlorophyll (BChl), two of the magnesium-deficient analogue, bacteriophaeophytin (BPh), one ubiquinone (UQ), one non-haem iron atom and three different proteins in a 1:1:1 ratio and of relative molecular masses 20000, 22000 and 28000 (Clayton 1973). These components are packaged in a lipid bilayer membrane with the primary UQ situated at or near the membrane surface with ready access to the aqueous medium. An 'artist's conception' of a possible arrangement is shown in figure 4. Reaction centres of *R. viridis* contain a similar complement of pigments and proteins, but the chlorophyll absorption bands are shifted to longer wavelength. The major band is at 960 nm in *R. viridis* compared with 870 nm in *R. sphaeroides*. RCs of *R. viridis* also retain bound cytochrome *C*, whereas *R. sphaeroides* RCs lose their cytochromes during the preparation procedure. This is an advantage for certain picosecond experiments, as will be seen later.

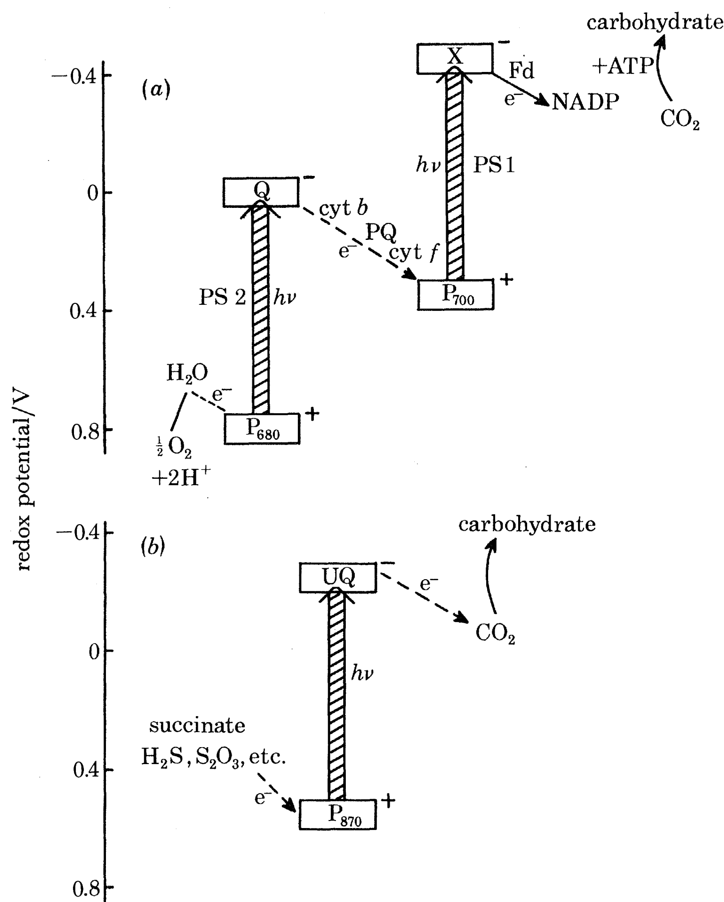

FIGURE 3. (*a*) Z-scheme for green plant photosynthesis; (*b*) similar scheme for bacterial photosynthesis.

4. Picosecond studies of electron transfer in bacterial reaction centres

4.1. *Rhodopseudomonas sphaeroides*

In RCs of *R. sphaeroides*, the longest wavelength absorption peak lies at 870 nm. In recognition of this the unexcited state of the RC is often characterized as P_{870} or, more simply, P. The absorption spectrum also displays bands near 540 and 760 nm attributable to BPh and bands

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near 600 and 800 nm attributable to BChl. The 870 nm band is strongly bleached upon illumination and a weaker absorption band characteristic of P^+ appears in the region of 1250 nm. This 1250 nm band can also be produced by oxidizing the RCs either chemically with, for example, potassium ferricyanide, or electrochemically. The 600 nm band bleaches to a lesser extent than the 870 nm band and the 800 nm band undergoes a slight blue shift to 798 nm. These changes, together with linear and circular dichroism measurements and e.s.r. studies, lead to the belief that two of the four BChl molecules form a closely coupled dimer or 'special pair' and that it is this species that, on oxidation, loses an electron to produce the primary oxidant P^+. The other two BChl molecules give rise to a component of the 800 nm band. The 540 nm band of BPh can be resolved at 77 K into two components at 532 and 544 nm, suggesting that the two PBh are situated in different environments (Clayton & Yamamoto 1976). See Holten & Windsor (1978) for a more detailed account.

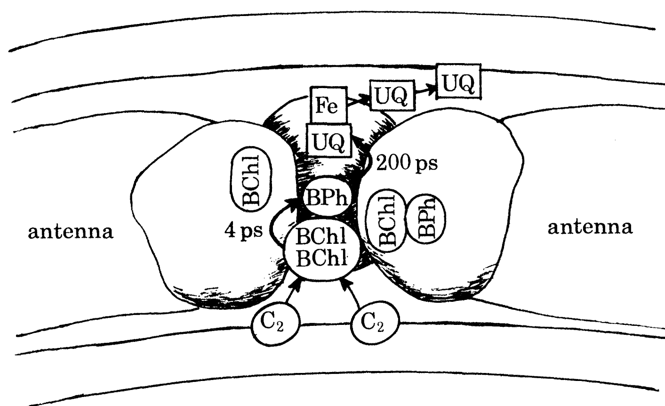

FIGURE 4. Artist's conception of bacterial reaction centre showing characteristic times of the early events in the primary charge separation.

The first attempts to observe transient intermediates in photosynthetic bacteria used RCs of *R. sphaeroides* poised at an artificially low redox potential by the presence of excess sodium dithionite (Parson *et al.* 1975). Lowering the redox potential in this manner, by reducing the UQ electron acceptor (often also denoted by X), would be expected to frustrate the transfer of an electron from any antecedent intermediate, thus increasing its lifetime. Indeed, with excitation by 20 ns laser flashes at 694 or 834 nm, the above authors observed the immediate (less than 20 ns) formation of a spectroscopic intermediate, which they called P^F, with an exponential decay time of about 15 ns. The quantum yield of formation of P^F is close to unity both at 295 K and at 15 K. As P^F decays, another intermediate, called P^R, appears which has a half-time of 6 μs at 295 K and 120 μs at 15 K. At 295 K its quantum yield of formation is low (0.1), but this value rises to unity at 15 K. The spectrum of P^R suggests that it is the triplet state of the BChl dimer. The processes described above are summarized in scheme (*b*) of figure 5. The other two schemes summarize the results of picosecond studies shortly to be described.

The rapid formation of P^F, and its accumulation in high yield when normal photochemistry is blocked, suggested that it might be an intermediate in the normal electron transfer reaction. But, then again, it might have been an artefact of the artificially reducing conditions used to facilitate its detection. To resolve this situation, studies of RCs under normal physiological conditions, i.e. with the acceptor X in its normal *unreduced* state, were needed. Picosecond laser

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studies provided the improved time resolution, Independent results from two laboratories (Kaufmann *et al.* 1975; Rockley *et al.* 1975) showed that in RCs of *Rps. sphaeroides* under these conditions, P^F could be spectroscopically identified. It appeared essentially immediately (less than 10 ps) on excitation with an 8–10 ps laser pulse, and exhibited a lifetime of about 200 ps. Kinetic studies showed that an absorbance decrease at 610 nm characteristic of P^+ appeared with the same time constant as the disappearance of P^F. These results demonstrated that P^F was not an artefact of the conditions used in the earlier nanosecond experiments, but was indeed a direct intermediate in the electron transfer reaction that culminates in the oxidation of P_{870} and the reduction of the acceptor X. They also showed that both steps in the two-stage transfer of the electron were exceedingly and perhaps unexpectedly rapid.

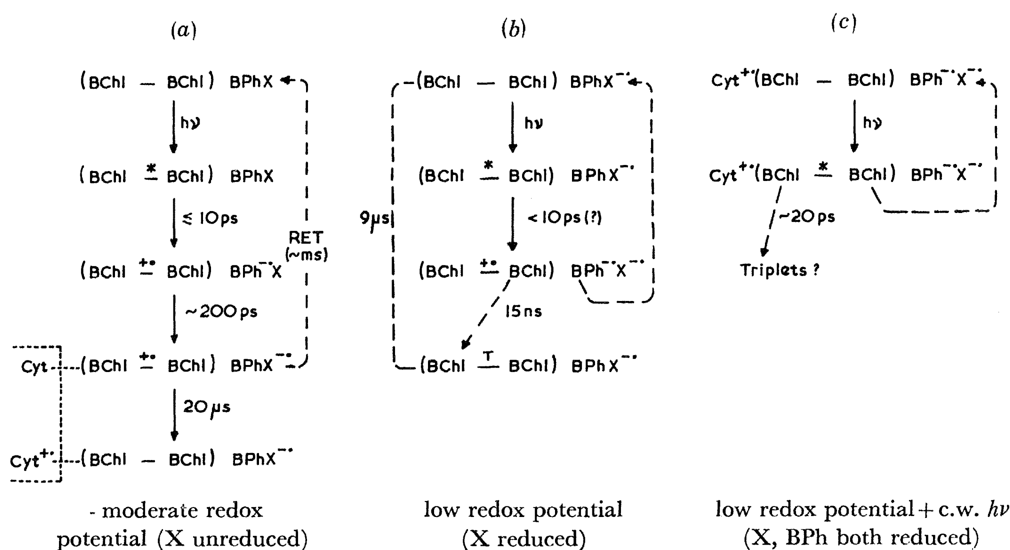

FIGURE 5. Scheme of early events in bacterial photosynthesis.

Attention now centred on the structural identity of the intermediate P^F. Rockley *et al.* (1975) compared their experimental spectrum with the sum of the absorbance changes that accompany the conversion of free BChl either to its cation radical, $BChl^{+\cdot}$ or its anion radical $BChl^{-\cdot}$. The calculated spectrum showed a fair measure of agreement, predicting the observed bleaching of bands near 380 and 600 nm and the appearance of new bands near 420, 500 and 650 nm, but did not predict the observed bleaching of the BPh bands. Such a procedure could be expected to give at best a rough approximation because it neglects interaction effects between the two molecules that form the biradical and also takes no account of the significant shift of the longest wavelength band of BChl from 770 nm in molecular solution to 870 nm in the RC. Nevertheless a surprising measure of agreement could be seen, as is shown in figure 6*a* and *b*. Subsequently Fajer *et al.* (1975) were able to obtain even better agreement by using a simulated spectrum composed of the sum of the absorbance changes that accompany chemical oxidation of RC (ΔP_{870}) and the reduction of BPh to $BPh^{-\cdot}$, which latter species had just been produced electrochemically and spectroscopically characterized in his laboratory. The comparison is shown in figure 6*c*. Further picosecond studies by Dutton *et al.* (1975) showed that the 1250 nm absorption band appeared promptly (less than 10 ps) upon excitation, rather than with the 200 ps risetime that would be characteristic of its formation from P^F. This led to the conclusion that the intermediate P^F must already contain the dimer cation radical P^+ as part of its structure, and lent

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further support to the assignment of Fajer *et al.* Thus it is now believed that P^F has the structure $(BChl)_2^{+\cdot}$ $BPh^{-\cdot}X$ and that the electron that takes up temporary residence on the BPh subsequently moves to X in a time of about 200 ps. These events are summarized in scheme (*a*) of figure 5.

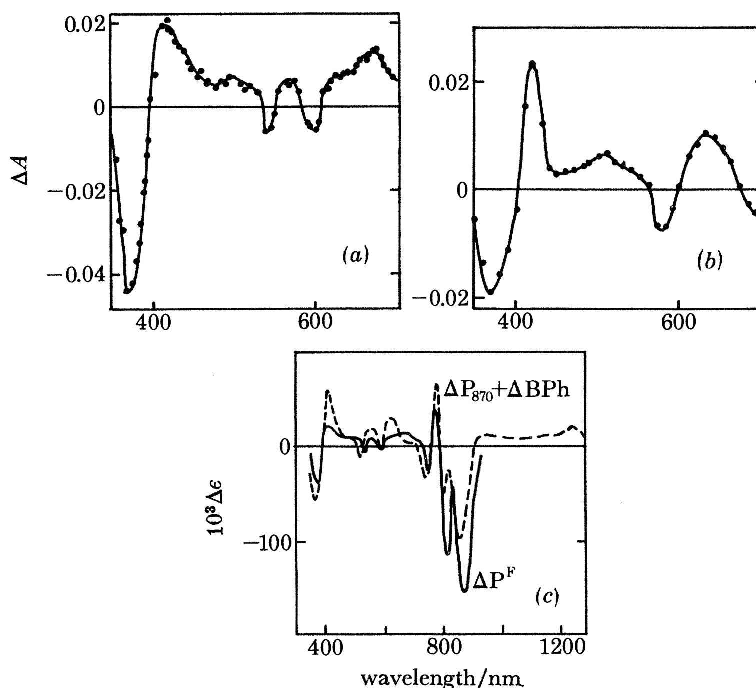

FIGURE 6. (*a*) Absorbance changes accompanying the photochemical formation of state P^F. (*b*) Sum of absorbance changes for $BChl^+$ and $BChl^-$ made electrochemically. (*c*) Comparison of laser-induced optical changes, ΔP^F, with sum of changes for ΔP_{870} plus ΔBPh^-.

Although much insight has been gained, the picture is still incomplete. Why has Nature placed a second BPh and two additional BChl molecules (the so-called P_{800} chlorophylls) in the RC? The low-temperature studies of BPh mentioned earlier suggest that only the longer-wavelength BPh is involved in the formation of P^F. Studies of *C. vinosum* (Tiede *et al.* 1976*a*), *R. viridis* (Tiede *et al.* 1976*b*) and *C. minutissimum* (Shuvalov & Klimov 1976) also support this conclusion. Perhaps the other two BChl molecules serve to 'solvate' one of the BPh molecules, as we have hinted in figure 4, thus facilitating the passage of an electron from $(BChl)_2$, or perhaps they help delocalize the transferred electron, thus hindering the back transfer. The first two of the above three studies cited also found partial bleaching at 800 and 595 nm, suggesting involvement of the P_{800} chlorophylls. It has also been suggested (Fajer *et al.* 1975) that the additional pigment molecules may have a role in coupling a part of the apparent energy losses that accompany the transfer of the electron to structural changes in the membrane that could, via Mitchell's chemiosmotic theory, be usefully harnessed to ATP production.

The identification of the acceptor X as ubiquinone (UQ) is supported by experiments by Kaufmann *et al.* (1976) showing that RCs depleted of UQ behave as though no acceptor is present. The state P^F lives for a time in excess of 1 ns. Addition of UQ restores the normal 200 ps kinetics.

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4.2. *Rhodopseudomonas viridis*

Since the RC of *R. viridis* retains bound cytochromes, it offered the intriguing prospect of artificially prolonging the lifetime of the excited singlet state of the special pair P* that presumably must precede the first step in the charge separation. The rationale was to place RCs of *R. viridis* not only at low redox potential to keep X reduced but also under conditions of continual illumination to saturate electron transfer and maintain I (a complex involving BPh)

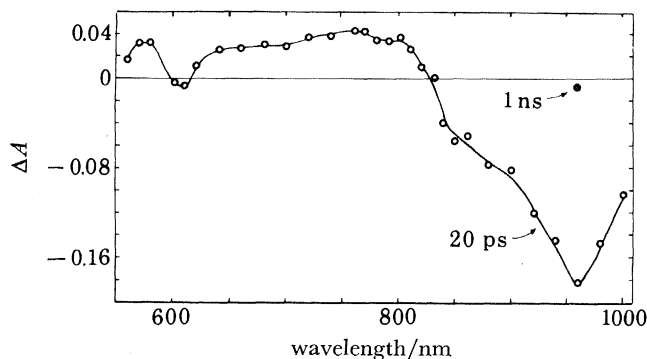

FIGURE 7. Difference spectrum accompanying formation of the excited singlet state, P*, of RCs of *R. viridis*.

also in the reduced state. The cytochromes would rereduce P^+ to P, leaving the system in the initial state, (BChl–BChl)-$BPh^{-\cdot}$ $X^{-\cdot}$, or more simply PI^-X^-, before picosecond excitation. Upon excitation, the reduced state of both I and X might be expected to frustrate electron transfer from P*, thus holding the system in the state $P^*I^-X^-$ for longer than usual. This scheme seems to have worked and we have observed, under these conditions, a spectroscopic intermediate that lives for about 20 ps (Holten *et al.* 1978*a*) (see figure 5*c*). At moderate redox potentials (figure 5*a*), corresponding to physiological conditions, the events observed closely parallel those found in *R. sphaeroides*. The P^F state is formed promptly and transfers an electron to X in about 230 ps. Netzel *et al.* (1977) found a prompt (less than 10 ps) rise of the 1310 nm band characteristic of P^+ in this species, in good agreement with our results. We also observe an additional step with 35 ps kinetics at 800–810 nm. At low redox potential (X reduced as in figure 5*b*), P^F lives for about 15 ns and decays, in part, to a longer-lived triplet state.

The difference spectrum corresponding to the conversion of P to P* under the 'super-reduced' conditions described above is shown in figure 7. The dominant feature is the bleaching of the 960 nm absorption band. Bleaching is present at 850 nm but drops to zero at 830 nm. The former band is characteristic of the special pair and the latter of the additional bacteriochlorophylls. These two bands are resolved in *R. viridis* but overlap in the 800 nm region in *R. sphaeroides*. These results support the conclusion that the primary donor in the RC is most likely the excited singlet state P* of a special pair of closely interacting BChl *b* molecules. However, interactions with the other two BChl *b* molecules are not excluded. E.s.r. and ENDOR studies (Fajer *et al.* 1977) suggest that the unpaired electron in P^+ is not shared equally by two identical molecules of BChl, as appears to be the case in *R. sphaeroides*. The spectral evidence also indicates that the intermediate acceptor I in *R. viridis* involves both BChl *b* and BPh *b* and that it is the BPh *b* component that is reduced when electron transfer from P* takes place.

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4.3. *Additional studies on RCs*

We have attempted recently to elucidate further the early stages of the charge separation process with the aid of mode-locked continuous wave (c.w.) dye laser equipment capable of subpicosecond time resolution and with provision for monitoring over a wide range of wavelength with the use of a subpicosecond continuum (Holten *et al.* 1980*b*). These new results indicate that after excitation of RCs of *R. sphaeroides* at 610 nm (a wavelength absorbed predominantly by the primary donor $(BChl)_2$), an absorbance increase occurs at 672 nm (characteristic of P^F) with a risetime of 4 ps or less. Thus the electron must move from the excited state of the special pair P* to BPh within 4 ps. This value of the transfer time is consistent with the less than 10 ps result obtained in the single-pulse laser experiments. An opposing view is, however, presented by recent experiments of Shuvalov *et al.* (1978) on RCs of *R. rubrum*. Using a 25 ps pulse at 880 nm for excitation, they reported that bleaching occurs at 800 nm within 15 ps of excitation, whereas no prompt bleaching of the BPh bands is observed. The 800 nm bleaching recovers with a lifetime of 35 ± 5 ps and is accompanied by the formation of BPh_- as shown by bleaching at 748 nm. The state P^+BPh^- subsequently decays in 250 ± 50 ps. From these results the authors conclude that the electron 'appears to be extracted from P by BChl-800 which gives a radical anion, BChl-800$^{-\cdot}$. Transfer of the electron to BPh then occurs in a time of about 35 ps'. Our own studies, while not ruling out the participation of BChl-800 as an intermediary electron carrier, indicate that any such involvement must take place on a time scale not greater than 4 ps, since transfer to BPh to form the radical pair P^+BPh^- is essentially complete within this time. Further studies with the shorter (0.7 ps) pulses provided by the mode-locked dye laser and at additional excitation wavelengths, including 800 nm, would be helpful. It is also desirable that a comprehensive series of measurements be made on a single species of bacterium. Because of the flexibility of making measurements under normal, reduced and super-reduced conditions of the UQ acceptor, *R. viridis* is probably the best choice for such studies.

4.4. *Summary of results on RCs*

The finding of similar transient intermediates with similar kinetics in several species of bacteria suggests strongly that the composition and structure of the bacterial reaction centre have evolved to maximize the efficiency of the primary charge separation process. Those species that have been subjected to detailed study seem to parallel each other closely with regard to the composition of their RCs. We propose to assume that the component pigments and proteins in the various RCs each have a functional role to play in the process of charge separation. In defence of this we argue that it is unlikely that 'unnecessary baggage' would have survived more than a thousand million years of natural selection. Given this assumption, we believe that further insight into how the RC functions and how its functioning is related to its internal structure requires studies of electron transfer in a variety of model systems that seek either to mimic or omit various features present in the RC.

5. Picosecond studies of electron transfer in model systems

We have shown (Holten *et al.* 1976) that electron transfer from bacteriophaeophytin (BPh) to *p*-benzoquinone (Q) in molecular solution takes place predominantly via the triplet state of the donor. Figure 8 shows that in the presence of excess methyl iodide, which enhances inter-

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system crossing i.s.c. in BPh by the heavy atom effect, the yield of the donor cation radical $BPh^{+\cdot}$ is greatly increased. Estimates of the half-cell potentials $BPh^{+\cdot}/BPh^*$ and $BPh^{+\cdot}/BPh^T$, the energy of the various collision complexes and the energy of the separated ions $BPh^{+\cdot}$ and $Q^{-\cdot}$ can be made (Gouterman & Holten 1977) and used to explain this observation (figure 9). In brief, we believe that both singlet and triplet charge transfer complexes $(BPh^{+\cdot}Q^{-\cdot})^S$ and $(BPh^{+\cdot}Q^{-\cdot})^T$ are formed from their respective collision complexes in less than 5 ps, but that the singlet complex suffers reverse charge transfer $(BPh^{+\cdot}Q^{-\cdot})^S \longrightarrow (BPh\ Q)$ in a time much shorter than that needed (*ca.* 230 ps) for the ion radicals to separate. The analogous process for the

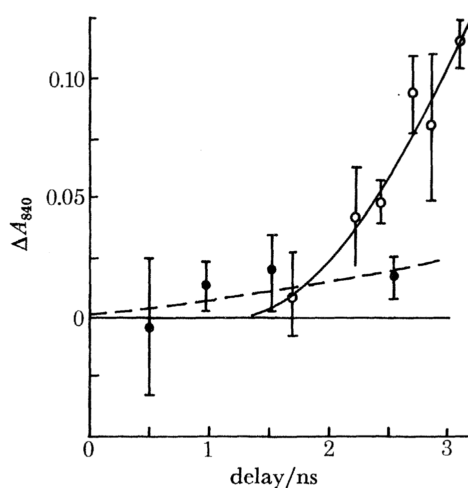

FIGURE 8. Enhancement of the yield of the cation radical $BPh^{+\cdot}$ by excess methyl iodide in the charge transfer quenching of BPh^* by *p*-benzoquinone. ○, With 8 M MeI; ●, without MeI.

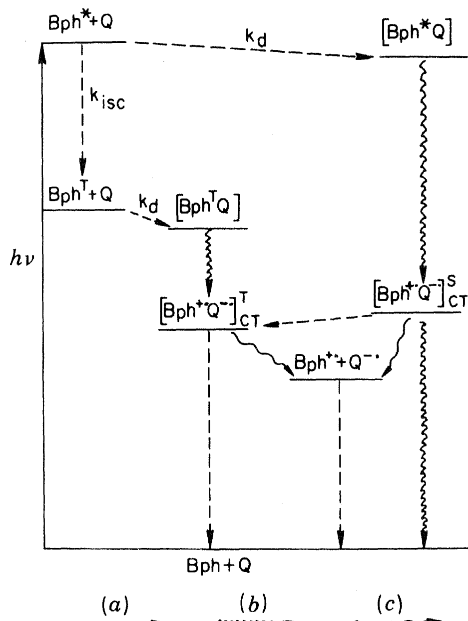

FIGURE 9. Proposed model for the bacteriophaeophytin (BPh), 40 mM *p*-benzoquinone (Q) and 8 M methyl iodide system, showing excited states and kinetic process. Here, k_d and k_{isc} are the rate constants for diffusion and intersystem crossing. The symbols S-CT and T-CT refer to singlet and triplet charge transfer complexes, respectively. (*a*) Processes slow because bimolecular or spin forbidden; (*b*) very fast spin allowed processes; (*c*) slower spin allowed processes.

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triplet complex is spin forbidden so that, in the triplet case, ion separation competes favourably with quenching. Similar experiments in which benzoquinone is replaced by methyl viologen (MV^{2+}) or by *m*-dinitrobenzene (*m*-DNB) give results that support the above model. For both these systems, formation of the radical ions is energetically possible only from the excited singlet state BPh* and not from the triplet BPh^T. For *m*-DNB, BPh* is quenched but $BPh^{+\cdot}$ is not detectable. For MV^{2+} the quantum yield of $BPh^{+\cdot}$ is reduced to about 10%. Were it not for Coulombic repulsion assisting the separation of the radical ions $BPh^{+\cdot}$ and $MV^{+\cdot}$ in this case, the yield would doubtless be much lower.

6. Theoretical implications for charge separation in photosynthesis

If efficient charge separation in the molecular systems discussed above can occur only via the triplet state of the electron donor, how can efficient electron transfer in the photosynthetic system proceed via the excited singlet state of the primary donor? Furthermore, an important difference between RCs and molecular solutions is that in the former the donor and acceptor are constrained by their association with other pigment molecules, e.g. the P_{800} BChls and with protein. The radical ions are not free to move apart as they are in solution. This means that discrimination against reverse electron transfer from $BPh^{-\cdot}$ to $(BChl)_2^{+\cdot}$ must be even more effective in the RC. To account for the observed 100% quantum yield of the charge separation process, it must be assumed that this back reaction is about 100 times slower than the next forward step that transfers the electron from $BPh^{-\cdot}$ to UQ. Since the latter takes about 200 ps, the back electron transfer from $BPh^{-\cdot}$ in the RC must take 20 ns or longer. This reverse transfer is close to 10^4 times slower than the forward step (*ca.* 4 ps) from $(BChl)_2$ to BPh.

A possible explanation, based on our model system studies (Holten *et al.* 1978*b*, *c*) rests on the size of the molecule that functions as the initial electron acceptor. A comparatively small molecule, such as the benzoquinone used in our quenching studies of BPh in solution, would undergo a significant shape change on formation of the charge transfer complex BPh^+Q^-. Good Franck–Condon overlap of the vibrational wavefunctions between the singlet charge transfer state and the ground state facilitates fast internal conversion of the approximately 1 eV of electronic energy separating the two states into vibrational degrees of freedom (see figure 10). Thus the charge transfer complex formed from the excited singlet state of the electron donor is deactivated before separation of the charged radical ions can take place. The same situation would apply in the photosynthetic RC were it not that Nature has contrived to place a comparatively large molecule, BPh, as an intermediate electron acceptor between the primary donor and the eventual small molecule ubiquinone acceptor. X-ray crystallographic data on some porphyrins and recent resonance Raman measurements on BChl and BPh indicate that addition or removal of an electron from these comparatively large molecules causes only quite small changes in bond length and bond angles. The corresponding Franck–Condon factors are small (figure 10), and the back reaction is effectively inhibited. The second forward step takes the electron in about 200 ps from BPh to UQ. A significant shape change is expected for the conversion of UQ to UQ^-. However, although the Franck–Condon factors are favourable, the back conversion of $(BChl)_2^+BPh\ UQ^-$ to the $(BChl)_2BPh$ UQ ground state of the RC complex would involve the movement over a considerable distance of the electron back through the BPh to the $(BChl)_2$. Thus at this juncture physical separation of donor and acceptor supervenes to inhibit the back transfer.

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The above model is speculative. Nevertheless it is valuable as a working hypothesis because it immediately suggests further experiments, the outcome of which may either disprove or modify the model. Of especial value would be studies of the quenching of BChl and synthetic BChl dimers in solution by acceptors of varying size. We plan to carry out such experiments when our instrumental developments make available suitable excitation wavelengths. An important step in this direction is the recent observation by Pellin *et al.* (1979) of a fast (less than 6 ps) high quantum yield electron transfer process in a model complex formed by covalently linking two molecules of pyrochlorophyll *a* with a primary alcohol derivative of phaeophytin *a*.

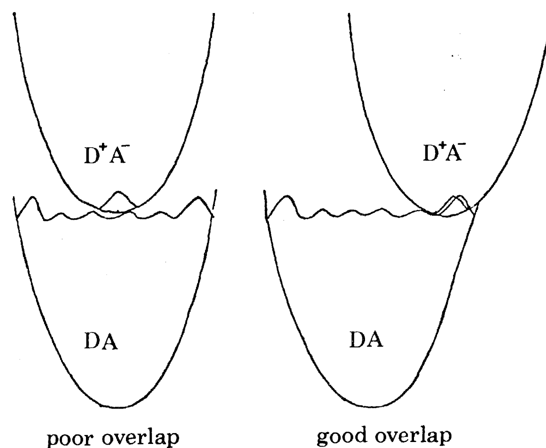

FIGURE 10. Franck–Condon diagrams for small shape changes (poor overlap) and large shape changes (good overlap).

The above model does not account for the extremely high speed (*ca.* 4 ps) of the initial electron transfer step from $(BChl)_2$ to BPh. Favourable π-orbital overlap between the $(BChl)_2$ and one of the BPh, assisted by a preferred mutual orientation, perhaps induced by the proteins to which the pigments are complexed, may be responsible. It can be argued that the high speed of this step probably evolved as a means of preventing the excitation from 'hopping back' into the antenna system. Efficient trapping of antenna excitation by the reaction centres requires rapid transfer, but this must be backed up by very fast initial charge separation to prevent back transfer.

In conclusion, it appears that in the bacterial reaction centre Nature has evolved an extremely efficient device for converting the energy of molecular electronic excitation into chemical potential via a multi-stage process of charge separation. This process relies on a very fast initial step and the indirect transfer of an electron to a desired acceptor via one or more intermediate sites. Together, these allow the transfer of an electron from a bacteriochlorophyll dimer to a ubiquinone acceptor without the fast back reactions that greatly reduce the efficiency in corresponding *in vitro* systems.

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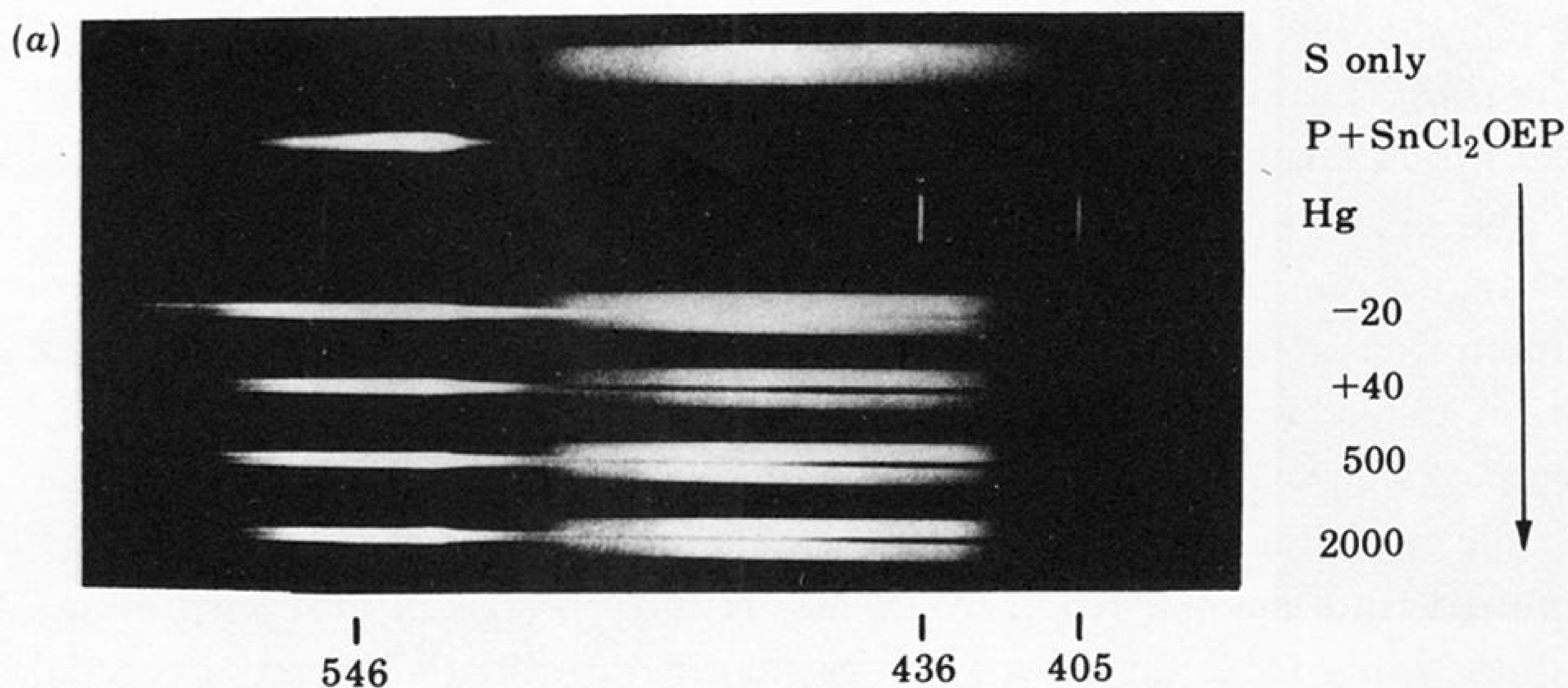

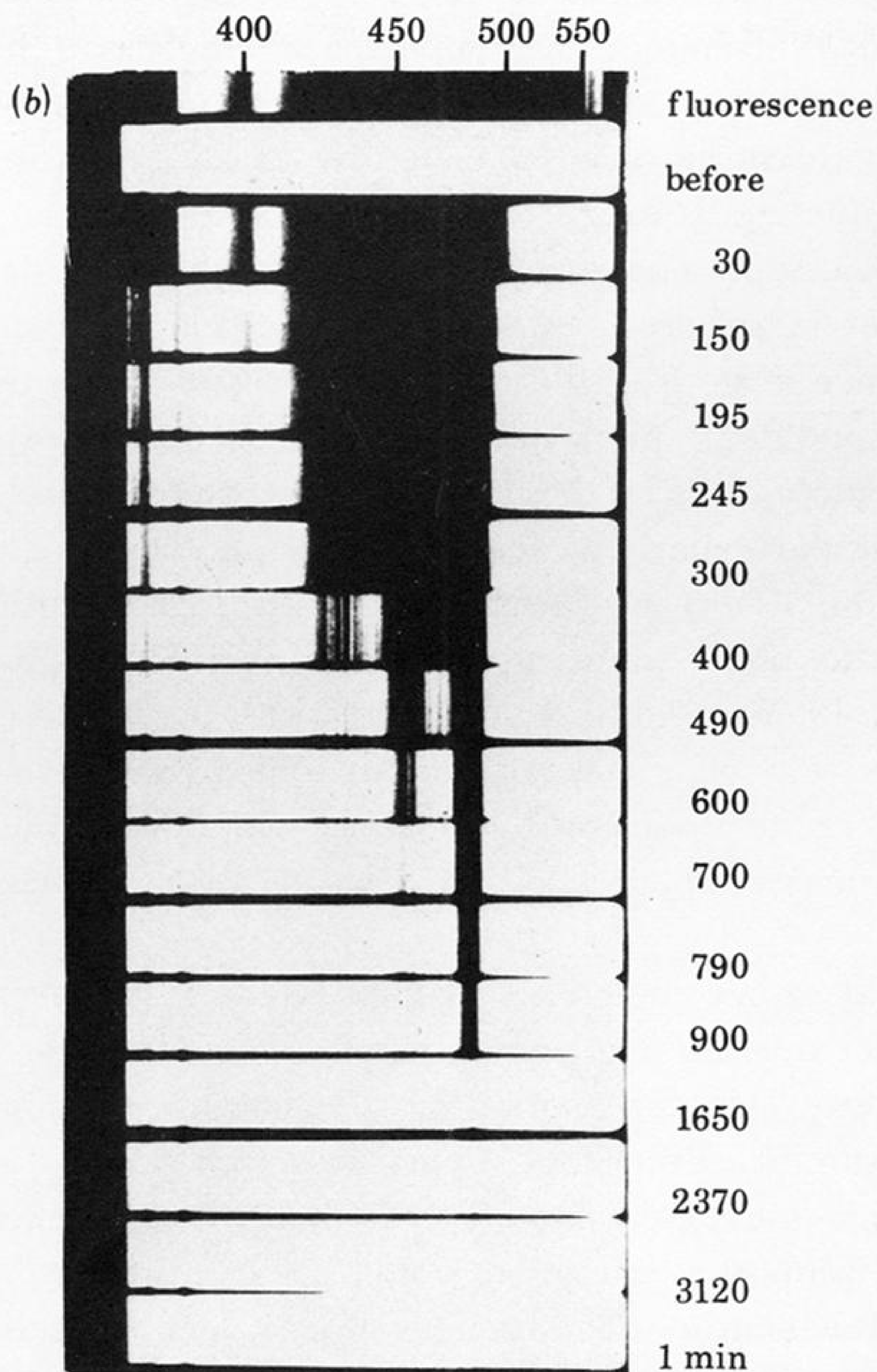

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IGURE 2. (*a*) Picosecond flash photolysis of tin dichlorooctaethyl porphyrin, showing decay of excited singlet absorption (short-lived) and triplet absorption (longer-lived). (*b*) Conventional flash photolysis of 1,2-benzanthracene in viscous paraffin solution showing decay of triplet absorption. Time scale in microseconds.