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Photochemical and Electron Transport Reactions of Bacterial Photosynthesis[1]

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INTRODUCTION

The past few years have been active and rewarding ones for those studying photosynthesis in bacteria, especially in the areas of the early photochemical events and related electron transfer and energy transfer reactions. The structure of the internal photosynthetic membrane systems of whole cells has received considerable attention, and the pioneering work has been done on the fragments produced from membranes by means of detergents. The primary phenomena of bacterial photosynthesis take place on the unique membrane system of the photosynthetic bacteria, and future work will directly relate to the general biochemical and physical properties of these membranes, with emphasis on the relation of ion transfer across membranes to the electron transfer and energy transfer processes which take place within the membrane proper. One can predict that studies conducted over the next few years will yield much information about the structure of the photosynthetic apparatus at the molecular and multimolecular level. Small, photoactive particles have been isolated from a number of bacteria (52–55). As described below, the isolation of a reaction center complex [containing the bacteriochlorophyll (Bchl) molecules which utilize the absorbed light energy to initiate the electron transfer reactions] has now been accomplished from *Rhodopseudomonas spheroides* chromatophores (103), and this important event will most certainly be followed by incisive experiments on the exact nature of the reaction center of the bacterial photosynthetic system.

I will not attempt to give a comprehensive review of all aspects of bacterial photosynthesis, but rather will restrict myself to the photochemical electron transfer reactions which take place in the membrane system, and attempt to relate these to the energy transfer reactions and structure of the membrane. Other sources that may be consulted as general references for the process of bacterial photosynthesis are found in references 20, 26, 27, 56, 65, 100, 119, and 122.

PRIMARY PHOTOCHEMICAL REACTION

Pigment Systems

The photosynthetic bacteria comprise three groups which are distinguished by the nature of the pigment (chlorophyll) they contain and the nature of the substrates they utilize. The green sulfur bacteria, or *Chlorobacteriaceae*, contain chlorobium chlorophyll and small amounts of bacteriochlorophyll-*a*, and their metabolism is centered primarily around sulfur compounds; hence, they are found in nature in environments which contain H_2S and are illuminated. The genera *Chlorobium* and *Chloropseudomonas* are found in this group. The purple sulfur bacteria, or *Thiorhodaceae*, contain Bchl *a* or Bchl *b*, are sulfur-oxidizing bacteria, and are represented by several species of *Chromatium* and *Thiospirillum*. In addition to their ability to utilize sulfur compounds, the photosynthetic sulfur bacteria also metabolize organic compounds quite readily. The nonsulfur purple bacteria, *Athiorhodaceae*,

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contain Bchl *a* or Bchl *b*, and are primarily dedicated to the metabolism of organic compounds. Examples of the latter group are the genera *Rhodopseudomonas*, *Rhodospirillum*, and *Rhodomicrobium*.

The chlorophylls occur in the photosynthetic bacteria in different combinations (91). Bchl *a* is found in most purple bacteria as the major pigment, and is also found in small amounts in several strains of the green bacteria. Structurally, Bchl *a* is quite similar to chlorophyll *a* of green plants. Bchl *b* is found in only a few purple bacteria and in a green photosynthetic rod (46, 64). The green bacteria have as the primary pigment a characteristic chlorophyll known as *Chlorobium* chlorophyll, of which several closely related types occur in a single bacterium along with a small amount of Bchl *a*.

In the purple bacteria containing exclusively Bchl *a*, the pigment occurs in the cell in different forms, which are recognized by the different absorbance maxima shown by the intact cell. Bchl *a* in organic solvents shows an absorption maximum in the region of 770 nm, whereas in the cellular environment absorption bands are noted in the regions of 800, 850, and 880 nm. The Bchl forms absorbing at these wavelengths are referred to as B800, B850, and B880. The difference in the absorption properties of Bchl in organic solvent and in vivo is due to the different environment in the cell, in which the Bchl is complexed either to a lipoprotein or to a carotenoid in the membrane, or is present as an aggregate of Bchl. In 1952, it was shown in the laboratory of Krasnovskii (72) that Bchl in solid films or in colloidal aggregates exhibited absorbance properties similar to those in the intact cells. Further work in 1967 (14, 73) concerned the effect of solvent vapors upon the Bchl absorbance properties, and this work showed that the bands observed at 800 and 860 nm for Bchl films are altered and a new band at 900 to 920 nm appeared in the presence of vapors of ether and another solvent such as water or methanol. Thus, it is possible to change the absorbance properties of isolated Bchl aggregates in a manner similar to that observed in vivo, which shows the sensitivity of the absorbance maxima of Bchl to the environment in which it is contained. The Krasnovskii group relates these data to an aggregated form of Bchl in vivo, since they can in part duplicate the absorbance phenomena with Bchl aggregates in vitro.

Chlorophyll-carotenoid interaction has been proposed by some as the cause for the absorbance shifts observed for Bchl in vivo (12, 60), but there is recent evidence against this explanation. It appears that the carotenoids influence the shifts only in the sense that lipids in general would have this effect, and no specific carotenoid-Bchl complexes are involved. Vernon and Garcia (120) have isolated a carotenoid-protein complex from *R. rubrum* chromatophores through the action of pancreatin and Triton X-100. This complex appears to be formed during the process of digestion, however, for the characteristic absorbance does not occur in the original membrane system.

There have been three protein-Bchl complexes isolated from the photosynthetic bacteria. One was obtained from the green bacteria *Chloropseudomonas ethylicum* and *Chlorobium thiosulfatophilum* by Olson (92). This complex contains Bchl *a* (the lesser component in these bacteria, whose main chlorophyll is *Chlorobium* chlorophyll) and shows an absorbance maximum in the infrared at 809 nm. This complex has been extensively studied by Olson, who has suggested that it might function in the bacteria as a reaction center complex, or at least be related to the reaction center of the bacterium. The small concentration of the Bchl *a* in these bacteria, combined with the longer wavelength absorbance of the Bchl, is consistent with this role, but to date no definitive photochemical role for this complex has been assigned. Two other Bchl-protein complexes were isolated by Vernon and Garcia (120) from *R. rubrum* by means of enzymatic digestion (pancreatin) in the presence of the detergent Triton X-100. One of the complexes was blue and exhibited absorbance maxima at 922, 835, 580, and 372 nm. The other complex was green and had absorbance maxima at 780, 587, and 360 nm. The green complex (but not the blue one) is active in simple photochemical electron transfer reactions. Both are soluble in water. Because of the prolonged treatment with the digestive enzymes required to form these complexes, it is doubtful that they represent the situation as it exists in the bacterial membrane. However, they do provide soluble complexes with which the properties of complexed Bchl can be extensively studied.

Reaction Centers

In 1952, Duysens (40, 42) showed that illumination of the purple photosynthetic bacteria caused a change in absorbance at 890 nm. This decrease in absorbance, or bleaching, was accompanied by another shift in the spectrum around 800 nm. These reactions have been studied in great detail by Duysen's group and by Clayton (25, 128). In 1960, Arnold and Clayton (2) reported that the reaction at 890 nm proceeded at 1 K, showing that thermal movement of the molecules was not involved in the reaction; rather, it must take place in a tight, well-ordered

complex. The Bchl responsible for the bleaching, and through which the photochemistry of the system is initiated, is called reaction center Bchl, abbreviated as P890 indicating that it is the photosynthetically active pigment which absorbs at 890 nm.

There is abundant evidence that P890 in *R. rubrum* (it is P870 in *Rhodopseudomonas spheroides*) is a Bchl in a special environment which in some manner confers upon it the property of being very reactive in electron transfer reactions, and which allows it to function as the initiator of the electron transfer reactions peculiar to photosynthesis. Light energy absorbed by other Bchl molecules is transferred to the reaction center Bchl for the initial photochemical event, which involves the transfer of an electron from the P890 to some adjoining molecule. The energy contained in the light quantum is thus transferred to the membrane in the form of chemical energy which is expressed by the distribution of electrons between the various compounds on the membrane. Therefore, we are immediately concerned with the oxidation and reduction reactions of these components, and it becomes the problem of the investigator to follow these reactions and deduce the sequence of electron transfer reactions which follow the absorption of the quantum of light.

The early experiments of Duysens and the later ones of Clayton showed a change in a Bchl which absorbs at 800 nm when chromatophores are illuminated. (The term "chromatophore" refers to the small vesicular membrane units which are obtained after rupture of the cell by either sonic oscillation or grinding. The photosynthetic machinery is contained on these small units which are apparently formed during disruption of the internal membrane system of the cell.) Clayton (30) has studied this change in *R. spheroides*, and has shown that it belongs to a Bchl molecule which is in a special relationship to the P870. The 800 nm form (called P800) always occurs in conjunction with the P870, and, when the bulk of the Bchl is destroyed by oxidation with iridic chloride, the resultant preparations show only the P800 and P870 (29). The band at 800 nm is 2.4 times as intense as the one at 870 nm. Illumination causes reversible bleaching (oxidation) of P870, which represents the initial electron transfer reaction of bacterial photosynthesis. Using differential extraction techniques, Clayton (29) showed that the treated chromatophores contained two molecules of P800 for every P870 molecule. The spectra of such chromatophores are shown in Fig. 1. Absorbed radiant energy is transferred more efficiently from the P800 to P870 than is light absorbed by

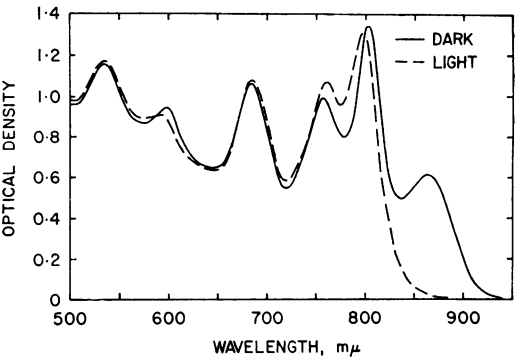

FIG. 1. *Absorption spectra of potassium iridic chloride-treated chromatophores of Rhodopseudomonas spheroides, with and without actinic illumination, showing the presence of both P800 and P870 and the photooxidation of the latter in the treated chromatophores. After Clayton (28).*

the bulk of the Bchl, which indicates a close association between the two special Bchl forms.

When a light quantum is absorbed by Bchl in the internal membrane system of a photosynthetic bacterium, the energy of the light quantum is transferred to the Bchl system, but is not localized in one particular Bchl. Rather, it is contained in a functional unit consisting of about 40 molecules of Bchl in the case of *R. rubrum* (28, 83), and this unit is serviced by one reaction center, P890. The absorbed energy activates the P890 to initiate an electron transfer reaction with surrounding molecules, one of which is an electron donor and another is an electron acceptor. The net result of the photoreaction is the transfer of an electron from the donor to acceptor, as discussed in a subsequent section.

Quinones as Electron Acceptors

There is good evidence that quinones function as electron acceptors in bacterial systems. The most common quinone in the photosynthetic bacteria is ubiquinone, which occurs with side chains of different lengths. Ubiquinone-10 is found in *Rhodopseudomonas capsulata*, *R. palustris*, *R. spheroides*, and *Rhodomicrobium vannielii*, whereas *Rhodopseudomonas gelatinosa* contains the quinone with eight isoprene units in the side chain, ubiquinone-8 (21). *Chromatium* strain D contains ubiquinone-7 (50), *Chromatium* 8379 contains ubiquinone-10 (21), and *C. vinosum* contains ubiquinone-8 (95). The concentration of ubiquinone varies in these organisms, but it is always a major component, reaching as high as one quinone to three Bchl molecules in the case of photosynthetically grown *Rhodospirillum*

rubrum (21). Furthermore, the quinone concentration is higher in the bacteria (*Athiorhodaceae*) when they are grown photosynthetically. Another quinone, rhodoquinone, is found in *R. rubrum* (59, 96, 111) and in *Euglena* (101). This quinone is structurally quite similar to ubiquinne, with the methoxy group on the 2 position replaced by an amino group (80). Takamiya et al. (111) have examined a large number of photosynthetic bacteria and algae, and they have reported that ubiquinone is the major quinone in all *Athiorhodaceae* examined, and that *R. rubrum* also contains rhodoquinone. *Chromatium* strain D contains vitamin K_2 in addition to ubiquinone and the green sulfur bacterium *Chloropseudomonas ethylicum* contains vitamin K_2 and a *Chlorobium* quinone-like substance Preliminary experiments performed in our laboratory (125) indicated that, in the case of *Rhodopseudomonas palustris* and *R. viridis* (originally described as *Rhodopseudomonas* species NHTC 133), a vitamin K-like substance is also present.

Because of their ubiquitous occurrence in high amounts in the photosynthetic bacteria, and because of their ready interaction with photoexcited chlorophyll in solution (106), quinones are very good candidates for interaction as an electron acceptor with reaction center Bchl. This reaction can be detected from absorbance changes in the ultraviolet region which result from the absorption of actinic light by the Bchl. Investigation of whole cells does reveal absorbance changes in the appropriate regions, but the direction of the changes indicates that the quinone may be either reduced or oxidized when the cell is illuminated. Redfearn (102) studied the redox state of ubiquinone in *R. rubrum* cells and chromatophores by extracting the cells and determining the extent of reduction of the quinone in the extract, and found that illumination caused an increase in the amount of the oxidized form. Parsons (98) measured the changes in absorbance for *R. rubrum* cells and showed that, whereas illumination caused a slow oxidation of the endogenous hydroquinone, the addition of phenylmercuric acetate changed the nature of the response so that a rapid reduction was observed upon illumination. Working with *Chromatium*, Takamiya and Takamiya (110) observed that under aerobic conditions the absorbance at 275 nm decreased, indicating a reduction of the quinone; this reduction amounted to about 7% of the total quinone. Adding $Na_2S_2O_3$ enchanced the change so the 25% of the quinone became reduced. Illumination under anaerobic conditions in the presence of malate, in which case the quinone would be more reduced prior to illumination, caused an oxidation of the hydroquinone form, which amounted to 50% of the total.

Whereas illumination of whole cells may cause either an oxidation or a reduction of the quinone, experiments performed on isolated chromatophores by following absorbance changes have routinely shown only a reduction of the endogenous quinone. Working with chromatophore films from *Chromatium*, *Rhodospirillum rubrum*, and *Rhodopseudomonas spheroides*, Clayton (24) showed that illumination caused absorbance changes in the ultraviolet region corresponding to the photoreduction of ubiquinone, which appeared to be coupled to the photooxidation of endogenous cytochrome. Also, Bales and Vernon (5) have demonstrated a photoreduction of ubiquinone coupled to the oxidation of the dye 2,6-dichlorophenol-indophenol (DPIP) by *R. rubrum* chromatophores. The reactions of endogenous quinone in *R. rubrum* chromatophores which had been treated with ferricyanide to oxidize the bulk Bchl have been determined by Beugeling (13), who showed a close relationship between the photoxidation of the reaction center P890 and the absorbance change thought to reflect the reduction of quinone. The amounts of P890 oxidized and ubiquinone reduced upon illumination were approximately equal, and had similar quantum requirements (0.8 to 1.3 quanta per molecule). Similar results were obtained by Ke et al. (66) on a small fragment produced from *Chromatium* chromatophores by the action of Triton X-100. This fragment contains the reaction center P890 and endogenous ubiquinone, and illumination causes an oxidation of the P890 and an apparent reduction of the quinone in a coupled reaction (Fig. 2). In this case, however, six quanta are required for the oxidation of one P890 molecule or the reduction of one ubiquinone. The primary nature of the reaction is indicated by the fact that it proceeds at the temperature of liquid nitrogen. The light-minus-dark difference spectrum shows only changes corresponding to P890 oxidation and ubiquinone reduction, and addition of ascorbate and phenazine methosulfate (PMS) at high concentrations (which allows the reduced PMS to react with oxidized P890) in the light results in a steady-state difference spectrum corresponding to ubiquinone alone. This is an important datum, since it shows that the absorbance change observed in the ultraviolet region with a trough at 275 nm is not due to P890 oxidation and is most likely due to ubiquinone. Recent experimentation with improved instrumentation on the same

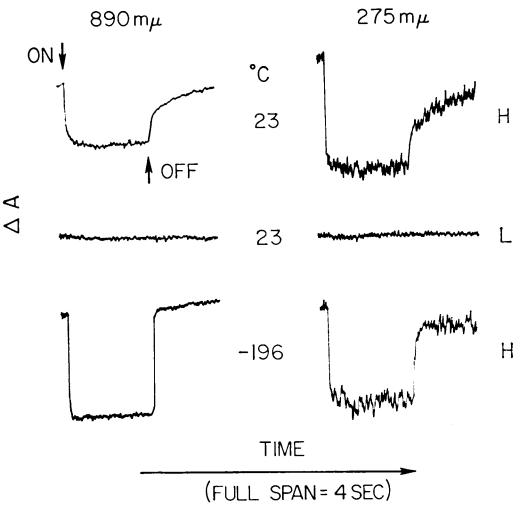

FIG. 2. *Light-induced absorbance changes corresponding to photooxidation of reaction center Bchl, P890, and reduction of ubiquinone (275 nm) in the small fragments produced from Chromatium chromatophores through the action of Triton X-100. The letter H refers to the heavy fraction obtained by density gradient centrifugation in sucrose, which is the small particle containing the reaction center; the L refers to the light fraction prepared in the same way. The L fraction is photochemically inactive. See reference 66 for more detail.*

particle from *Chromatium* shows that the reaction times of the P890 and ubiquinone are equally fast, and are as fast as 1 μsec (B. Ke, *personal communication*).

The data cited above indicate that there is a reduction of endogenous quinone coupled to the oxidation of reaction center Bchl, but there are two data that are not explained by this simple interpretation. The first problem discussed above related to the fact that both quinone reduction and reaction center Bchl oxidation have the same quantum requirement in the systems investigated to date. This would not be expected, since the quinone change is a two electron reaction while P890 oxidation involves only one electron. The second problem relates to the fact that the electron spin resonance (ESR) signal observed with the bacterial species investigated cannot be related to a quinone radical. This would indicate that the transition from the radical form (UQH) to the fully reduced form (UQH_2) is very rapid (less than 1 msec). If the second electron came from a molecule other than the Bchl, the difference in the quantum requirements could be explained. Much more work needs to be done in this important area.

All the data obtained for ubiquinone changes are consistent with the quinone acting as an electron acceptor for the reaction center Bchl. This reaction could be a direct one, or some unknown intermediate compound could intervene. Since the P890-ubiquinone coupled reaction with *Chromatium* particles proceeds at 77 K, the quinone and P890 must be closely coupled in order for the electron transfer to take place in the frozen system. It is still possible, however, that some other intermediate compound could intervene. To date, however, the known compound which functions closest to the reaction center Bchl as an electron acceptor is ubiquinone.

Chromatophores of the photosynthetic bacteria (*R. rubrum*, *Chromatium*, *R. spheroides*) are also capable of reacting photochemically with added exogenous quinones, as shown by the experiments of Zaugg et al. (134). With ubiquinone-2 or ubiquinone-6 as the electron acceptor and the reduced forms of DPIP, PMS, or N,N,-N′,N′-tetramethyl-*p*-phenylenediamine (TMPD) as the electron donors, very rapid electron transfer reactions were observed upon illumination. A similar reaction is observed when reduced mammalian cytochrome *c* is used as the donor molecule (133). These experiments show that the isolated chromatophores have the ability to couple with added ubiquinones with shorter side chains (which are more water-soluble), but the reaction is also catalyzed by isolated Bchl which is solublized by the presence of asolectin (134). Further experiments of this type were performed by Redfearn (102), who showed that the reaction was not specific for ubiquinone derivatives, since other benzoquinones were also active in the reaction with reduced cytochrome *c*. Later experiments by Zaugg et al. (135) showed that the $PMSH_2$-ubiquinone photoreaction is not coupled to adenosine triphosphate (ATP) formation, and therefore does not involve the endogenous electron transfer components of the chromatophore which are involved in the cyclic electron transport system which couples to ATP formation. Since this reaction would not be limited in rate by the usual dark enzymatic steps encountered in the in vivo system of the chromatophore, it would be expected to proceed at high rates, and would also not be coupled to ATP formation.

Ferredoxin

Another compound which is a candidate for the role of electron acceptor for the reaction center Bchl is ferredoxin. In plants, ferredoxin serves the role as the first stable photoreduced product, and it in turn leads to the reduction of nicotinamide adenine dinucleotide phosphate

(NADP) (104). In the chloroplast system, however, there is good evidence that another compound, called X, functions between ferredoxin and the reaction center chlorophyll *a*, P700 (45, 70, 123), and its photoreduction has escaped detection to date. In bacteria, also, ferredoxin appears to be a normal constitutent of the photosynthetic system. It has been detected in *R. rubrum* (109), has been isolated and crystallized from *Chromatium* (4), and has been purified from *R. palustris* (132) and *Chlorobium thiosulfatophilum* (47). The protein isolated from *R. palustris* has most of the properties of a ferredoxin, but has a higher midpoint potential that the other ferredoxins isolated from the photosynthetic bacteria. It appears to be intermediate between the ferredoxins and the high-potential iron proteins isolated from *Chromatium* (39) and *R. gelatinosa* (37).

To date, it has not been possible to show a requirement for ferredoxin isolated from a photosynthetic bacterium for the photoreduction of nicotinamide adenine dinucleotide (NAD) by that bacterium. Experiments have been performed with *R. rubrum* (85) and *Chromatium* (62), but the ferredoxin from *R. palustris* was not tested in a photochemical system (132). Therefore, there is no direct evidence of a role for bacterial ferredoxin in NAD photoreduction. The ferredoxin from *Chlorobium thiosulfatophilum*, however, is reduced photochemically and supports the fixation of carbon dioxide into pyruvate and α-ketoglutarate (48) in a newly discovered system of carbon dioxide fixation in the photosynthetic bacteria.

Recent studies have shown that 2-amino-4-hydroxy pteridines are associated with the photosynthetic apparatus in a number of bacteria (51, 79, 86). Since the presence of inhibitors of pteridine formation also causes an inhibition of photophosphorylation and carbon dioxide fixation in these organisms (86), it is possible that the pteridines could serve as electron acceptors for the reaction center Bchl. Their low potential makes this hypothesis attractive, since in the reduced form they could transfer electrons to either ferredoxin or ubiquinone, but to date there has been no definitive evidence for such a role. Isolated bacterial chromatophores do react with a number of artificial electron acceptors, however, including oxygen (75, 114), quinones (133, 134), methyl viologen (coupled to a disulfide as the final acceptor) (82), and methyl red (3).

Cytochrome c as Donor for Reaction Center Bchl

In 1953, a cytochrome *c* was observed in *R. rubrum* (113, 115), and Duysens observed that this cytochrome became oxidized in the light (41). Since that time, numerous cytochromes of the *c* type have been isolated from the photosynthetic bacteria (11), and it is apparent that cytochromes of this type are found in all photosynthetic bacteria. Cytochromes of the *b* type are also present, and it is commonly held that these cytochromes have the same relationship in the chromatophores that they do in other electron transfer systems; i.e., they form an electron transfer couple which functions in the photosynthetic cyclic electron transfer system which leads to ATP formation (119, 124). Whereas in mitochondrial electron transfer systems the cytochrome *c* transfers electrons to cytochrome oxidase and thence to oxygen, in the photosynthetic bacteria cytochrome *c* transfers the electron to photooxidized P890. Examples of photooxidation of cytochrome *c* are too numerous to be discussed in detail here. Some representative papers on the subject are discussed in reference 121.

The cytochromes are photooxidized with quantum requirements of 0.7 to 1.4 for *Chromatium* and 3 to 4 for *R. rubrum* (1). This indicates a very efficient reaction for the oxidation of the endogenous cytochrome. Evidence that the cytochrome and P890 form a complex that efficiently transfers an electron from the cytochrome to the oxidized P890 was obtained by Chance and Nishimura (22), who showed that with *Chromatium* the photooxidation of cytochrome 423.5 (one of four cytochromes oxidized) proceeds at 77 K with an efficiency about equal to that at room temperature. The cytochrome was not reduced during a subsequent dark period. On this basis, it was proposed that the primary photoreaction of bacterial photosynthesis was the transfer of an electron from the complexed cytochrome to the reaction center Bchl. A subsequent study by Vrendenberg (130) has shown that, with other bacteria studied, cytochrome photooxidation decreased as the temperature was lowered, and the photooxidation stopped at different temperatures. Apparently, the highly efficient cytochrome oxidation shown by *Chromatium* is unique, and these data indicate that cytochrome oxidation is not necessarily the first reaction of photosynthesis. This will be discussed in more detail below.

Since the original discovery by Olson (88) that cytochromes in chromatophores of *Chromatium* are oxidized by light, there has been intense interest in this organism. Based on a series of studies by his group, Duysens (45) has concluded that in the case of *Chromatium* there are two cytochromes of the *c* type involved in the photo-

chemical reactions, but these serve different functions. One of these, called cytochrome $c_{423.5}$ (the number indicates the maximum for the light-induced difference spectrum), functions in the cyclic electron transfer system characteristic of all photosynthetic bacteria, while the other functions in the terminal system through which electrons are fed from the substrate molecules into the photochemical system. The two cytochromes are experimentally distinguished by their different reaction kinetics and by their oxidation rates in air. Similar experiments with *R. rubrum* have been performed by Sybesma and Fowler (108), who also propose the presence of two photosystems corresponding to these two functions of the cytochrome *c*. Also, by determination of action spectra for the photooxidation of the two cytochromes of *Chromatium*, Morita (81) shows that the various forms of Bchl in this bacterium are effective to different degrees in causing the oxidation of these cytochromes. From these data, he proposes the presence of three separate photochemical systems in this organism. Cusanovich et al. (36) have studied particle preparations from *Chromatium*, and have proposed the existence of two separate electron transfer systems in this bacterium, one driven by P890 and another driven by a reaction center Bchl absorbing at 905 nm. Thus, it is evident that the cytochromes of the *c* type can be experimentally distinguished according to function in the photosynthetic apparatus, and it appears that these cytochromes are also in different environments as far as the Bchl system is concerned.

The oxidized form of the reaction center Bchl, P890, reacts readily with electron donors other than endogenous cytochrome *c*. The experiments of Zaugg et al. (133, 134) show that the reduced forms of PMS and TMPD couple efficiently with oxidized P890. Further evidence relative to TMPD is available from experiments relating to the light-induced ESR spectrum (61), and experiments showing the effect of PMS on either the light-induced P890 absorbance changes or the ESR signal show that the reduced form of this compound donates electrons directly to oxidized P890 (33, 66, 97).

Primary Photochemical Act

As discussed above, two consequences of the absorption of a light quantum by the photosynthetic systems of bacteria are the subsequent reduction of endogenous ubiquinone and the oxidation of endogenous cytochrome. Which of these is the primary act? Considering the quinone as an acceptor (*A*) and the cytochrome as a donor (*D*), the following two possibilities exist:

$$(1)\ D\cdot P890\cdot A \xrightarrow{\text{light}} D\cdot P890^{+}\cdot A^{-} \rightarrow D^{+}\cdot P890\cdot A^{-}$$

$$(2)\ D\cdot P890\cdot A \xrightarrow{\text{light}} D^{+}\cdot P890^{-}\cdot A \rightarrow D^{+}\cdot P890\cdot A^{-}$$

In case 1, the photoexcited P890 reacts initially with the acceptor to produce an oxidized P890, while in case 2 the P890 reacts first with the donor molecule to produce a reduced P890. The available evidence indicates that mechanism 1 above is the correct one, for the following reasons.

Bleaching of the reaction center Bchl, P890, represents an oxidation of this Bchl moiety, since similar absorbance changes can be brought about by the addition of oxidizing agents such as ferricyanide to the chromatophore system (25, 27). A reduction of the P890 could also cause a loss of absorbance of P890, but there is no direct experimental evidence that such a reaction takes place.

One of the initial responses of the bacterial photosynthetic system to light is the production of some compound with unpaired electrons, which results in the observations of a light-induced ESR signal. According to Loach et al. (76, 77), this light-induced ESR signal is related to the oxidation of P890 in a one-electron oxidation, at least in the case of *R. rubrum* chromatophores. An extensive study of the light-induced ESR signal from *Chromatium* cells and chromatophores conducted by Schleyer (105) led to the conclusion that the ESR signal was produced by a component of the photosynthetic electron transfer system of this bacterium. The signal was shown by the organized photosynthetic systems in both the cell and the chromatophore, and was also shown by Bchl in solution. The compound which is responsible for these light-induced responses shows a midpoint potential of 0.44 v (76) which is in agreement with its function as the reaction center Bchl. In the oxidized form, it could receive electrons from the reduced cytochromes whose potentials generally fall in the range of 0.29 to 0.33 v (11).

The characteristics of interaction of phenazine methosulfate with illuminated chromatophores are consistent with its interaction with the oxidized form of P890. Cost et al. (33) have reported that this compound causes an increase in the decay rate of the photo-produced ESR signal and the related absorbance change. The data of Zaugg et al. (134) show that the reduced form of PMS reacts with both reaction center Bchl and Bchl in solution.

The data of Ke et al. (66) show that with the small particle isolated from *Chromatium* chromatophores the absorbance changes related to

the photoreduction of endogenous ubiquinone take place in a coupled reaction with P890. The experiments reported allowed a comparison of the rise times of these reactions, from which it was concluded that they were faster than 50 msec. Later experiments (B. Ke, *personal communication*) show that these reactions are both faster than 1 μsec. There is no cytochrome oxidation with this particle, and when the reduced form of PMS is added the decay rate of the P890 absorbance change is drastically increased, so that at higher PMS concentrations there is no observable absorbance change at 890 nm. At the same time, the absorbance changes due to ubiquinone continue. These data indicate a direct transfer of electrons to the quinone as the first photoreaction.

Definitive data on the initial photochemical reaction are furnished by the experiments of Parsons (97, 99), who used a 30-nsec flash from a ruby laser to initiate the photochemical events. With *Chromatium* chromatophores, the oxidation of P890 was accomplished in less than 0.5 μsec, and the presence of dithionite abolished the reaction (presumably by reducing the acceptor quinone). The oxidized P890 recovered in 2 μsec, and parallel experiments showed that the cytochrome c_{422} became oxidized at the same rate, indicating that the P890 donated its electron to an adjoining acceptor molecule in the initial photochemical reaction and subsequently received an electron from the adjoining cytochrome in a secondary reaction. In experiments with *R. rubrum*, it was found that the initial reaction was complete in less than 0.2 μsec. Thus, in both bacteria, the oxidation of P890 is the primary event.

In considering the compounds which might serve as the electron acceptor for photoexcited P890, ubiquinone and ferredoxin are the logical candidates. The latter is reduced photochemically by *Chlorobium thiosulfatophilum* (48) and is utilized in carbon dioxide reduction. The fact that a number of the photosynthetic bacteria evolve hydrogen gas upon illumination on appropriate substrates (119) shows that the bacteria can operate at the low potential required for hydrogen evolution. However, to date there is no evidence that ferredoxin can be reduced in a direct reaction with the reaction center Bchl. There is evidence that ubiquinone is reduced in a reaction that is primary (13, 66). This will be discussed in more detail below.

Secondary Biochemical Reactions

Once the initial photochemical step is accomplished, the subsequent reactions are biochemical in nature and consist of a series of electron transfer reactions through a complex of compounds contained in the bacterial membrane system. Accompanying this electron transfer process is the formation of ATP; the process is called photophosphorylation. In principle, then, this system should be like the membrane system of mitochondria and of chloroplasts, which also carry out electron transfer reactions and an associated phosphorylation. Figure 3 presents in

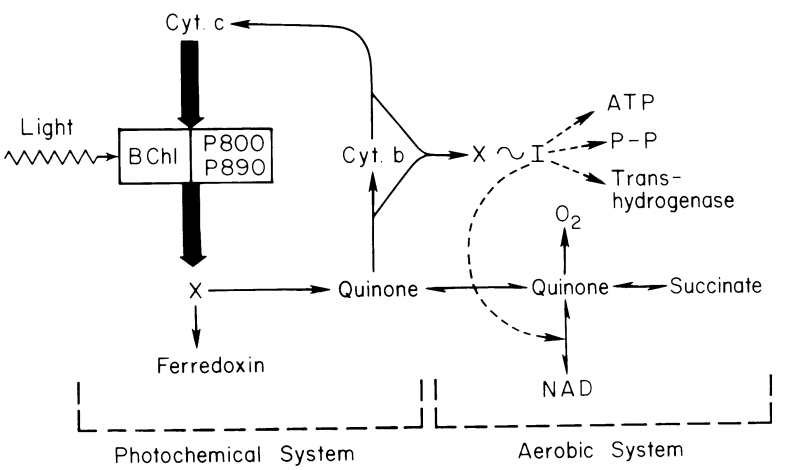

FIG. 3. *Electron transfer pathways in Rhodospirillum rubrum. The movement of electrons through the cyclic pathway, driven by the initial photochemical reaction of P890, produces the high energy state, X ~ I, which may be used as indicated. The photoreduction of NAD which is observed with isolated chromatophores is driven by the high-energy state, and for reasons given in the text is portrayed as resulting from the aerobic metabolic system which is present in this bacterium.*

schematic form the components of the bacterial electron transfer system, but more specifically it pertains to *R. rubrum*. The scheme shows a cyclic system containing the Bchl in three forms, the bulk Bchl, the reaction center P890, and the P800 which always accompanies the P890. This system serves to capture the light energy and initiate the electron transfer process by the process described above. In addition to the Bchl system, the cyclic system is shown as containing ubiquinone, cytochrome *b*, and cytochrome *c*, as well as an unknown compound X which serves as the initial electron acceptor. There is not space in this review to consider the evidence placing the cytochromes in this cyclic system, and the reader is referred to references 119 and 121 for more information. The positions of cytochrome *c* and ubiquinone are discussed above, and X is included in the scheme for the following two reasons. In experiments currently underway with the small particle obtained from *Chromatium* by the action of Triton X-100 (66), under some experimental conditions it is possible to observe a light-induced oxidation of P890 with no accompanying observable reduction of the ubiquinone, indicating that some other compound is serving as the electron acceptor. Secondly, in the case of *Chlorobium thiosulfatophilum*, ferredoxin is photoreduced by the chromatophore system, showing that it is possible to photoreduce this low-potential compound in a system which does not photoreduce NAD, making it highly likely that the ferredoxin is reduced by direct electron transfer from the Bchl system via X. Ferredoxin is not listed as the initial acceptor, since in all experimental procedures tried to date, in which either Bchl or Chl has been used, ferredoxin does not act as an electron acceptor for the photoexcited chlorophyll in a manner similar to quinones. Furthermore, all evidence points toward a similar situation in the plant chloroplast, where the P700 chlorophyll *a* photoreduces some compound prior to ferredoxin (123).

Also shown in Figure 3 are the components of the aerobic electron transfer system which is found in *R. rubrum* cells. This bacterium has the ability to grow in the dark by virtue of an oxidative metabolism, although this supports a rather slow growth and only low rates of oxygen utilization are observed. This system is depicted as being coupled to the photochemical cyclic system in two ways: via reversible electron transfer reactions between the quinones and by the high-energy state produced during energy transfer reactions leading to ATP formation. It is not known whether these two systems are contained on the same membrane system, or whether they are on separate systems. The coupling of the two systems via the high-energy state, X$\sim$I, is discussed below in relation to the mechanism of NAD photoreduction. The separate nature of the quinones in the two systems is illustrated by the fact that the difference spectrum obtained for the reduction of endogenous quinone by light differs from that obtained by the chemical reduction with succinate (98). The photoreduction of NAD by this bacterium could be accomplished either by using succinate to feed electrons into the system as shown or by using other donors (including artificial ones such as DPIP or TMPD) to feed electrons into the photochemical system.

NAD Photoreduction

In those cases where a photoreduction of a nicotinamide nucleotide has been shown, NAD is the nucleotide reduced preferentially by bacteria. Although a photoreduction of NAD has been shown for *Chromatium* chromatophores, it is limited and has not been studied to the extent that the reaction in *R. rubrum* has (62). A photoreduction of NAD with chromatophores of *Rhodopseudomonas capsulata* has been reported by Klemme and Schlegel (69), with rates up to 50 μmoles per hr per mg of Bchl reported. This photoreduction resembles that observed for *R. rubrum*, in that suitable donors for the reaction are succinate, reduced DPIP, and hydrogen. The effect of added ferredoxin on this reaction was not reported. In the case of *R. capsulata* (69), *R. spheroides* (94), and *R. rubrum* (112), NADP is also photoreduced, but this reaction is coupled via a transhydrogenase to the initial photoreduction of NAD.

The photoreduction of NAD has been studied most extensively with *R. rubrum* cells and chromatophores. A more detailed review of the earlier experiments of this nature is given in references 119 and 118, so these experiments will be given only a cursory treatment here. Duysens and Sweep (43) and Olson et al. (89) followed NAD photoreduction in *R. rubrum* and *Chromatium* cells by means of the increase in blue fluorescence which results from this reaction. Duysens (44) and Amesz (1) extended these studies with *R. rubrum* and *R. spheroides* whole cells, showing that a high rate of NAD photoreduction was observed for short periods after the onset of illumination, giving a rate of 360 μmoles of NAD photoreduced per hr per mg of Bchl. This rate is considerably faster than that observed for intact chromatophores, which at best give rates of 50 (same units).

The photoreduction of NAD by isolated chromatophores of *R. rubrum* is supported by those

electron donors which can interact with the chromatophore electron transfer system. Frenkel (49) used reduced flavin mononucleotide (FMN) as the donor, whereas in the early experiments of Vernon (116, 117) succinate was used as the donor. Nozaki et al. (84, 85) used ascorbate and DPIP as the donor system in their series of experiments. The simplest interpretation of the photoreduction observed with chromatophores was that the electron donor (succinate, $DPIPH_2$, etc.) was donating electrons for the electron transfer system, and the electrons were then transferred via photoexcited Bchl to the NAD. This was the interpretation placed on the reaction by the early investigators, and was used as the basis for Nozaki et al. to distinguish between cyclic and noncyclic photophosphorylation in the bacterial system by analogy to the plant system. Gest (16, 57) proposed that the so-called noncyclic photophosphorylation in *R. rubrum* was actually of the cyclic type, and was proceeding simultaneously with the net electron transfer from the donor to the acceptor, and that, furthermore, the cyclic electron transfer system functioned in the reaction to produce ATP which then drove the electron transfer reaction in an energy-requiring step. Thus, with succinate as the donor, the succinate-to-NAD electron transfer is an energy-requiring reaction, and the energy for the step is provided by the ATP produced by cyclic photophosphorylation.

Recent experiments on isolated *R. rubrum* chromatophores have shown that at least some, and most likely all, of the observable NAD photoreduction proceeds via the reverse electron flow mechanism. The most convincing datum supporting this concept is the demonstration of a succinate-supported reduction of NAD by chromatophores in the dark when ATP is supplied. Löw and Alm (78) reported in a 1964 abstract that this reaction had been noted, but it was described in detail by Keister and Yike (68). Although the rate of the dark ATP-driven reaction is low by comparison with the photoreduction (we have never observed it to exceed one-third of the light-induced rate), the effects of inhibitors of electron transport and uncouplers are consistent with the concept that both go by similar mechanisms. Uncouplers of phosphorylation inhibit both reactions, whereas inhibitors of electron transport peculiar to the cyclic electron transport system inhibit only the light-driven reaction. Oligomycin stimulates the light-driven reaction while it inhibits the ATP-driven reaction. The addition of a phosphate acceptor system (adenosine diphosphate plus inorganic phosphate) inhibits the light-driven reaction, and this inhibition is overcome by the addition of oligomycin.

Keister and Yike propose that the NAD reduction, both the light-driven and the ATP-dependent type, is accomplished by the aerobic electron transport system found in *R. rubrum* cells. It is for this reason that Fig. 3, which was adapted from that presented by Keister and Yike (68), shows the NAD reduction system as separate from the cyclic electron flow system. This would be consistent with the fact that appreciable rates of NAD photoreduction are observed only with bacteria which have an aerobic metabolism, such as *R. rubrum* and *R. capsulata* (although *R. spheroides* does not show NAD photoreduction). The photoanaerobe *Chromatium*, which shows a photoreduction of NAD in experiments conducted with intact cells, shows only a very low rate of NAD photoreduction with chromatophores, and this could be related to the very slight aerobic metabolism carried out by this organism (58).

Since the rate of NAD photoreduction by intact *R. rubrum* cells is far greater than is observed with isolated chromatophores, and since that observed by the chromatophores is most likely carried out by the aerobic metabolism component of the bacteria, we must still consider the possibility of a direct photoreduction of NAD by the photochemical system of the bacteria, which could be routinely inactivated through the process of rupturing the cells and preparing the chromatophores. The ability of *Chlorobium thiosulfatophilum* chromatophores to photoreduce ferredoxin, and the inability of other photosynthetic bacteria to do so, although ferredoxin is present in the cells, indicates that this ability is probably lost or masked during the preparation of chromatophores of these bacteria.

Trebst et al. (112) have studied the effect of an inhibitor of ferredoxin reduction for plant chloroplasts, disalicylidenepropanediamine disulfonic acid, upon the photoreduction reactions of *R. rubrum*. Whereas this inhibitor is effective with plant chloroplasts, it has no effect upon NAD photoreduction by *R. rubrum* chromatophores. Also, the addition of ferredoxin to the NAD photoreduction system has no effect. These data are in accord with the conclusion that the NAD photoreduction of *R. rubrum* involves the aerobic system, and it is driven by the high-energy state, $X\sim I$, formed by cyclic electron flow as shown in Fig. 3.

Another important reaction involving the nicotinamide nucleotides in bacterial photosynthetic systems has recently been reported by Keister and Yike (67), who demonstrated that *R. rubrum* chromatophores have an energy-requiring transhydrogenase (the enzyme which transfers electrons between NADH and NADP), and that this enzyme can be activated either by

light (which produces the high-energy state via electron transfer reactions) or by the addition of ATP or of pyrophosphate. The equivalence of ATP and pyrophosphate in *R. rubrum* chromatophore reactions will be discussed below. A similar reaction has been reported for *R. spheroides* chromatophores by Orlando et al. (94), but in this case pyrophosphate could not be used as an energy source to drive the reaction.

Photophosphorylation and Energy Transfer

Since the formation of ATP accompanying the photosynthetic electron transfer reactions is almost universally observed, it is reasonable to assume that it occurs in all photosynthetic systems, and, in cases where it is difficult to demonstrate, some operational obstacle for its exhibition is present. There is considerable literature on the subject of bacterial photophosphorylation, and the interested reader is referred to previous reviews and articles for background material (16, 56, 57, 85, 119). The outline of the process is shown in Fig. 3, which indicates that there are two sites of ATP formation in the cyclic electron transfer system. The presentation of two sites for ATP formation is based on some early experiments from the laboratory of H. Baltscheffsky. In collaboration with Arwidsson (7), he observed that the antibiotic valinomycin inhibited ATP formation in *R. rubrum* chromatophores to the extent of only 50%, which they interpreted to mean that one of two phosphorylation sites was available to valinomycin.

A second reason for thinking in terms of two phosphorylation sites is the fact that the inhibitors antimycin A and HOQNO affect the cyclic electron transport system of *R. rubrum* chromatophores and completely stop the cyclic phosphorylation process. The addition of PMS or DPIP to the inhibited system allows phosphorylation to proceed again (see reference 119 for pertinent references). Since PMS is thought to react with oxidized P890 (33, 66, 97, 135) and the inhibitors are thought to act at the cytochrome *b* level, this means there must be a phosphorylation site on the electron transfer span which is not bypassed by the PMS, or at the quinone level. This is substantiated by the experiments of Baltscheffsky et al. (6), who reported that the quantum requirement for ATP formation in *R. rubrum* chromatophores was approximately 6 quanta per ATP formed; in the presence of antimycin A and PMS, the requirement went up to about 10 quanta per ATP formed. These data indicate that one site is inhibited and nonfunctional in the presence of antimycin A, and that the other site is carrying out the ATP formation under these conditions. The site functioning under these conditions is presumably located at the quinone level in Fig. 3. Further evidence for separate sites of phosphorylation in *R. rubrum* chromatophores is provided by the experiments of Horio and Yamashita (63), who showed that irradiation with ultraviolet light or treatment with Triton X-100 distinguishes between the phosphorylation catalyzed by added ascorbate and that stimulated by added PMS.

Another expression of the energy-conservation mechanism of *R. rubrum* chromatophores is the formation of pyrophosphate, P-P, coupled to photosynthetic electron transport (8, 9). In this reaction, the activated phosphate intermediate is transferred to a phosphate molecule instead of adenosine diphosphate, so that pyrophosphate results. This reaction represents another way in which the activated state produced by electron transport may be energetically utilized; also, the use of pyrophosphate provides another way of producing the high-energy state in bacterial chromatophores. M. Baltscheffsky et al. have used both ATP and pyrophosphate to produce the high-energy state in *R. rubrum* chromatophores, and have shown that this is then utilized to cause a reduction of cytochrome *b* (9, 10). Addition of these compounds causes an increase in absorbance at 423 nm, which is interpreted to mean a reduction of cytochrome *b*. These data indicate that cytochrome *b* is involved in ATP formation in this bacterium, and would locate one of the two phosphorylation sites at the cytochrome *b* level, or at the cytochrome *b*-cytochrome *c* couple. This site would be the one inactivated in the system inhibited by antimycin A in the presence of PMS. Using the same technique, absorbance changes corresponding to the carotenoid compounds can be observed, which would indicate that the transformation responsible for the carotenoid shift is also intimately related to the phosphorylation mechanism.

Following the observation that light causes an uptake of protons into chloroplasts, bacterial chromatophores were examined to see whether a similar reaction occurred. Subsequent to the early observation of Chance et al. (23), others have studied this reaction (34, 126, 127). The magnitude of the reaction observed with *R. rubrum* chromatophores is less than that observed for spinach chloroplasts. The quantum yield of proton movement into the chromatophore is at best 0.1 proton moved per quantum (23), which is considerably less than the value of 5 protons moved per quantum absorbed by spinach chloroplasts (38). On the basis of these data, Chance et al. concluded that proton movement per se was not involved in a direct way in the transfer of energy from the electron transfer system to

ATP formation, but was rather a secondary reflection of some phase of the ATP formation.

There remains considerable work to be done before the proton change induced by light in bacterial chromatophores can be related in a mechanistic way to the electron transfer reactions and ATP formation. Whether the proton gradient so produced is the energetic force which drives ATP formation is neither proved nor disproved. The proton movement is obviously closely related to the ATP-forming system, since the proton gradient is strongly affected by uncoupling agents. von Stedingk and Baltscheffsky (126) reported that, whereas gramicidin inhibits the proton change in *R. rubrum* chromatophores, valinomycin in the presence of potassium ion stimulates the proton movement. A significant study made recently in the C. F. Kettering Laboratory by Shavit et al. (107) revealed that the antibiotic nigericin in the presence of potassium was a potent uncoupler of phosphorylation in *R. rubrum* chromatophores, and under the proper conditions it inhibited the proton pump while leaving ATP formation not significantly affected.

STRUCTURE

There has been considerable interest within the past few years in the structure of the photosynthetic apparatus in bacteria, with emphasis upon the use of detergents to fragment the membrane into smaller units which can be studied for their composition and photoactivity. The treatment of bacterial systems with detergents began with the observations by Komen that sodium dodecyl sulfate produced changes in the location and intensities of the Bchl absorption bands of *R. rubrum* and *Chromatium* chromatophores (71). Subsequently, Brill (17) used the detergent Triton X-100 to convert *R. spheroides* chromatophores into two fractions, one enriched in the longwave Bchl form (B880) and the other enriched in the two shorter wavelength forms B850 and B800. (These terms denoting the location of the three main Bchl absorption bands are used for all bacteria considered, although in some cases the actual absorption maxima may differ from these wavelengths.) Brill later studied the effects of deoxycholate upon *Chromatium* chromatophores (18, 19) and showed that a bleaching of the B850 occurred. In this case, energy transfer between the Bchl forms was inhibited, which is another indication of a structural alteration caused by the detergent. Clayton performed experiments on *Chromatium*, and showed that deoxycholate produced two fragments, and one was enriched in the B880 component.

We have begun a study of the fragmentation of the purple photosynthetic bacteria with Triton X-100, and have studied the fragments so produced in some detail. A general treatment of these investigations has appeared (125), and more specific papers have been published which contain the details for the experiments with *Chromatium* (52), *R. rubrum* (53), *R. palustris* (54), and *Rhodopseudomonas* sp. NHTC 133 (*R. viridis;* 55). (For a general treatment of the internal structure of the photosynthetic bacteria, see reference 32.) Chromatophores from the purple bacteria examined are fragmented into two fragments which are separable by density gradient centrifugation. One of the fragments is a small particle, and the other one is more membranous in nature, indicating that the detergent removes a portion of the photosynthetic system in the form of the small particles, leaving the residuum intact.

A comparison of the properties of the fragments produced by Triton X-100 is shown in Table 1. The fragments are designated as "heavy" or "light" depending upon their relative positions after sucrose density gradient centrifugation. The small particle released by the detergent may be either the light or the heavy fraction, and in some (but not all) cases there is a separation of the Bchl forms between the two fragments. Electron micrographs of the various fractions obtained from these four bacteria are contained in reference 125. The bacteria examined to date fall into one of two groups. The first one, including *Chromatium* and *R. palustris*, have the photochemically active particle in the heavy fraction, and there is a clear separation of the Bchl forms between the two fragments. Only the small particle carries out the primary photochemical reactions, as shown by the oxidation of the reaction center Bchl and the reduction of endogenous ubiquinone. The other group includes *R. rubrum* and *R. viridis*, which yield two fractions that do not differ appreciably in Bchl content. The light fraction contains the photoactive particle, but the photooxidation of reaction center Bchl also takes place in the other fragment, which is more membranous in nature.

The data obtained in our laboratory indicate that the detergent Triton X-100 solubilizes a portion of the bacterial membrane system, liberating small particles into the medium. In the cases we have examined, this particle contains the reaction center Bchl and the long wavelength Bchl (B880). This shows a clear distinction in the environment of the various Bchl forms in these bacteria. The small particles liberated vary in size and in their tendency to aggregate. In the case of *R. palustris*, the particles tend to

TABLE 1. *Properties of the subchromatophore particles prepared from four species of photosynthetic bacteria through the action of the detergent Triton X-100*[a]

Chromatophore fragment	Appearance	Bchl content[b]		Reaction center Bchl[c]	Endogenous UQ photoreduction[d]	Fast ESR signal	Quinone content[e]
		A_{880}/A_{800}	A_{880}/A_{850}				
Chromatium		(0.58)	(0.60)				
Heavy	Particulate, 0.7 pm	3.4	3.1	+	+	+	UQ
Light	Membranous	0.12	0.24	Absent	Absent	Absent	UQ
Rhodopseudomonas palustris		(1.14)	(0.9)				
Heavy	Particulate, in strands 0.65 to 0.80 pm wide	2.4	1.3	+	+		UQ (6.7), vitamin K-like
Light	Membranous	0.63	0.56	Absent	Absent		UQ (5.4), vitamin K-like
Rhodospirillum rubrum		(4.7)					
Heavy	Membranous	3.5		+	+	+	UQ (2.8)
Light	Particulate, 0.5 pm	3.6		+	+	+	UQ (3.4)
Rhodopseudomonas NHTC		(6.7)					
Heavy	Aggregated particles (1.3 pm spacing)	8.0		+	Absent		UQ (1.6), vitamin K-like
Light	Particles, some aggregated, 1.35 pm	8.0		+	+		UQ (1.4), vitamin K-like

[a] The designation of heavy and light fractions refers to the relative position following a sucrose density gradient centrifugation. UQ represents ubiquinone.

[b] The absorbancies (800, 850, and 880 nm) refer to the general locations of the three forms of Bchl. The exact locations in each case were as follows: *Chromatium*, 890, 850, and 800 nm; *R. palustris*, 873, 857, and 802 nm (805 in chromatophores); *R. rubrum*, 877 and 800 nm; and *Rhodopseudomonas* NHTC, 1,005 (1,015 in chromatophores) and 830 nm. The values in parentheses are for chromatophores.

[c] The reaction center Bchl was measured at the following wavelengths; *Chromatium*, 890 nm; *R. palustris*, 870 nm; *R. rubrum*, 890 nm; and *Rhodopseudomonas* NHTC, 940 nm.

[d] Determined by absorbance change at 275 nm.

[e] The numbers in parentheses are the ratios of Bchl to UQ on a molar basis. Similar data are not available for *Chromatium*, but ubiquinone is present in approximately equal amounts on the two fractions. The vitamin K-like quinone migrates in the region of vitamin K during thin-layer chromatography, but has not been positively identified.

form predominantly linear aggregates 6.5 to 8.0 nm thick. Some linear aggregates are also found with *R. viridis*, but more often the aggregates are of a two-dimensional planar nature. Where tests have been made, the cytochromes are found in this fraction, although it has not been possible to demonstrate a photooxidation of the cytochromes. Perhaps the cyclic electron transfer system has been disrupted so that only the initial electron transfer from the reaction center Bchl to the adjoining ubiquinone molecule is seen.

The plant chloroplast system is similarly affected by the detergent Triton X-100 (123, 125), yielding two fractions which are representative of the two photosystems operative in plant photosynthesis. Whether Triton X-100 or digitonin is used for the fragmentation process, the small particulate fraction so produced contains photosystem 1, shows the photooxidation of P700 (the reaction center Chl *a* in plants), and carries out a photoreduction of NADP. This is the plant counterpart of the bacterial small particle. In the plant system 1 particle, there is a

photochemical transfer of an electron from the P700 reaction center Chl *a* to some acceptor, X, and thence to ferredoxin. The analogy between the plant and bacterial systems carries over to the photooxidation of P890 and a photoreduction of ubiquinone. This points out a similarity between the plant and bacterial systems in terms of their component parts and general reactions, both photochemical and biochemical. In both cases, the complete photosynthetic apparatus includes distinct units which are imbedded within and form part of the membrane system. The membrane continuum contains the shorter wavelength chlorophyll forms, which in the case of bacteria consist of the accessory Bchl forms which are designed to capture the light quanta and transfer the energy so obtained to the reaction center where the reduction of NAD (leading to CO_2 fixation) takes place. In the case of the plant chloroplasts, also, the membrane continuum contains the shorter wavelength-form of Chl *a* of photosystem 2, which leads to the evolution of oxygen. This photosystem is accessory, however, since its function is to provide electrons to photosystem 1 so that NADP photoreduction (and carbon dioxide fixation) can proceed. Since substrate molecules furnish the electrons necessary for carbon dioxide fixation in bacteria, there is no need for the elaborate biochemical machinery required to oxidize water; therefore, there is no need for the separate photoact that is seen in plants, and the shorter wavelength Bchl serves only in a light-harvesting capacity.

The successful isolation of the reaction center from *R. spheroides* has recently been reported by Reed and Clayton (103), who used Triton X-100 to fragment the chromatophores into three fractions which were separable by density gradient centrifugation on a discontinuous sucrose gradient. One of these fractions contained the reaction center Bchls, P800, and P870, but no B870. Another fraction was devoid of the reaction center Bchls but contained the light-harvesting Bchl, B870. These data show that the reaction center exists as a separate entity in the chromatophore membrane. Extensive studies of the characteristics of light absorbance changes and the related fluorescence changes (31, 128, 129) show that the reaction centers exist separately in the membrane and are fed absorbed light energy by the Bchl system which forms the bulk of the membrane system. The reaction centers are not independent in terms of accepting energy, but rather form an integrated system with the light-harvesting Bchl continuum so that light absorbed by the bulk Bchl is available to a number of reaction centers. Clayton concluded (31), "Energy transfer extends over a number of P870 molecules, as if the tissue contains an extended matrix of light-harvesting pigment studded here and there with photochemical reaction centers." Our data are certainly in agreement with this concept.

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