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PHOTOSYNTHETIC MEMBRANE DEVELOPMENT IN  
*RHODOPSEUDOMONAS SPHEROIDES*: INCORPORATION OF  
BACTERIOCHLOROPHYLL AND DEVELOPMENT OF ENERGY  
TRANSFER AND PHOTOCHEMICAL ACTIVITY

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SUMMARY

1. Bleached cells of *Rhodospseudomonas spheroides*, obtained by aerobic growth in the light, rapidly synthesize bacteriochlorophyll and carotenoids when transferred to 4% oxygen in the dark. Fluorescence excitation spectra of whole cells during the initial stages of pigment synthesis indicate that the major fraction of the newly synthesized bacteriochlorophyll is immediately incorporated into photosynthetic membranes and can receive excitation energy transferred from the carotenoids. Activity of the photosynthetic reaction centers, as measured by the effect of dithionite addition on the fluorescence of the incorporated bacteriochlorophyll, develops simultaneously with pigment incorporation.

2. Fluorescence excitation and emission spectra also indicate the presence of an unbound form of bacteriochlorophyll *in vivo* as well as a form of bacteriopheophytin. We suggest that this free bacteriochlorophyll is in a "pool" from which it is incorporated into the membranes and that the bacteriopheophytin (or bacteriopheophorbide) is formed by the degradation of the free and thus unprotected form of the bacteriochlorophyll.

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INTRODUCTION

MÜLLER *et al.*<sup>1</sup> have found that the fluorescence lifetime of chlorophyll *a* in *Chlorella* is 0.35 nsec at low light intensity, compared to 2.0 nsec in inhibited cells and 5.0 nsec in ether<sup>2</sup>. In our opinion a high degree of spatial organization at the reaction center is required to achieve this rapid ( $k \approx 3 \cdot 10^9/\text{sec}$ ) utilization of energy. The ultimate objective of our research is to understand what this organization is and how it is achieved.

Photosynthetic bacteria have already provided a great deal of information on the type of interactions which occur at the reaction center. One of the most promising lines of research has been that of CLAYTON (see REED AND CLAYTON<sup>3</sup>) who has obtained preparations from *Rhodospseudomonas spheroides* in which only the reaction center pigments, P870 and P800, remain. SAUER *et al.*<sup>4</sup> have suggested, from studies

of the circular dichroism spectra of such preparations, that the reaction center in *Rps. spheroides* consists of a trimer of bacteriochlorophyll molecules. The nature of other molecules in this complex is obscure.

It has long been known that certain members of the Athiorhodaceae can be grown aerobically and that under these conditions they synthesize little or no photosynthetic pigment<sup>5</sup>. Pigment synthesis can be induced in aerobically grown cells by transfer to anaerobic conditions in the light or to low oxygen tensions (less than 5% O<sub>2</sub>) in light or dark<sup>6</sup>. The classic paper of COHEN-BAZIRE *et al.*<sup>6</sup> has been the basis of all subsequent work in this area. Many other aspects of pigment development have been studied<sup>7-15</sup>, and the process appears to involve the induction of biosynthetic enzymes *via* mechanisms similar to those observed in other bacteria.

Our principle concern with this process has been the details of the incorporation of bacteriochlorophyll into the photosynthetic membranes. We feel that such a developmental approach will complement the degradative approach of Clayton and others and provide new information on the interactions at the reaction center. In preliminary reports, we have shown that bacteriochlorophyll can be detected in bleached cells of *Rps. spheroides* within 30 min after transfer to semi-aerobic conditions, and our data suggest that the bacteriochlorophyll becomes photosynthetically functional within 1-1.5 h after transfer<sup>16</sup>. A second pigment component, fluorescing at 790 nm *in vivo* rather than at 885 nm characteristic of the light-harvesting bacteriochlorophyll in these cells, was observed during the early stages of pigment synthesis, and it was tentatively concluded that this component was bacteriochlorophyll or a precursor which was not yet incorporated into the membrane<sup>17</sup>. In this paper we present additional data which support these conclusions and demonstrate the simultaneous incorporation of carotenoids into the functional photosynthetic unit. In addition we shall discuss the implications of our data for the nature of the biosynthesis of the membrane and photosynthetic apparatus.

#### MATERIALS AND METHODS

Cells of the green mutant of *Rps. spheroides* (strain 2.4.1/Ga) were grown in modified Hutner's medium<sup>6</sup> which is equally suitable for aerobic, semi-aerobic, and photosynthetic growth. The initial depigmentation of the cells was carried out by aerobic growth in the light in volumes of 10-15 ml. Under these conditions, vigorous bubbling with air provides both adequate mixing and aeration of the entire culture. After 15-20 generations of such growth, at which time no bacteriochlorophyll was detectable by our sensitive fluorescence method, a logarithmic phase culture was used to inoculate a 200-ml volume in a gas wash bottle equipped with a sintered glass aerator (Corning No. 31770). This culture was allowed to grow in a water-bath at 29-30° in the light under vigorous bubbling with air (80 cm<sup>3</sup>/min). The culture was also stirred rapidly with a magnetic stirrer, in order to facilitate equilibration between the gas and the liquid phases. When the absorbance\* of the culture at 950 nm was approx. 0.1, the light was turned off and the aeration switched to 4% oxygen (semi-aerobic conditions). The 4% oxygen was obtained by mixing air and nitrogen (20:80, by vol.) in a Matheson gas proportioner, at a total flow rate of 80 cm<sup>3</sup>/min. Samples

\* The light intensity is diminished due to scatter by the sample rather than to absorption.

were removed from the culture at intervals by means of a tube and syringe system without opening the culture vessel or interrupting the gas flow, and the  $A_{950\text{ nm}}$  was determined. Each sample was then divided into two 3.0-ml portions and centrifuged at  $0^\circ$  by acceleration to  $23000 \times g$  and immediate deceleration in a Sorvall RC2B centrifuge. The cells from one of these portions were resuspended in 3.0 ml of 0.05 M potassium phosphate buffer (pH 6.8), and fluorescence excitation or emission spectra were obtained immediately, both before and after the addition of  $50\ \mu\text{l}$  of a 0.1 g/ml solution of sodium dithionite. The second portion of cells was put into a freezer immediately after centrifugation to await extraction with 3.0 ml of a methanol-acetone mixture (2:7, by vol.) and recentrifugation as above to remove the cellular debris. The fluorescence emission intensity at 785 nm of this cell-free extract was then utilized as a measure of the total cellular bacteriochlorophyll content.

The fluorescence spectra were obtained by using an EMI 9684A photomultiplier tube cooled with dry ice and a Jena interference-wedge filter (model Veril SL-200, bandpass about 14 nm) together with a scanning drive, power supply, amplifier, and recorder. Excitation was obtained from a quartz-iodine lamp and passed through a dilute  $\text{CuSO}_4$  solution and a Jarrell-Ash 0.25 m Ebert monochromator with 2 mm slit width (bandpass about 9 nm) (see Fig. 1). The wedge scanning drive consists of a stepping motor (Hayden No. K44139) driven by a Heath multispeed drive unit (EUA-20-26). This motor can be run in synchrony with the chart drive on a Heath recorder (EUA-20M) such that the scanning speed can be varied while maintaining a constant ratio between the chart and wedge movement. This was used to obtain emission spectra with a constant excitation wavelength. For excitation spectra, the monochromator scan was driven with a synchronous motor at a rate of 100 nm/min, with a constant chart speed on the recorder and with the wedge set at the desired wavelength of emission.

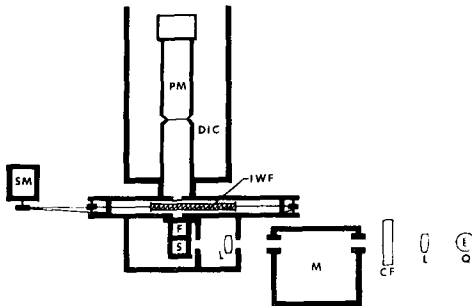


Fig. 1. Schematic diagram of apparatus. CF, copper sulfate filter; DIC, dry-ice chamber for cooling the photomultiplier; F, chromic acid filter; IWF, interference-wedge filter; L, lens; M, monochromator; PM, photomultiplier; S, sample; SM, stepping motor; Q, quartz-iodine lamp.

The spectra have not been corrected for either the variation in incident intensity as a function of wavelength or the sensitivity of the photomultiplier-wedge combination. Since most of the data are presented either as ratios or as a developmental sequence which has been scanned through the same wavelength region, the correction for the combination is essentially a constant and the fact that it has not been employed does not affect the interpretation of the data.

## RESULTS

Fig. 2 shows the growth rate and development of pigmentation when an aerobically grown, bleached culture in the light is switched to semi-aerobic conditions in the dark. The growth rate, as measured by the increase in absorbance at 950 nm, remains constant and is unaffected by the change in oxygen tension or light intensity. Bacteriochlorophyll, as measured by either fluorescence at 885 nm *in vivo* or fluorescence at 785 nm in a methanol-acetone extract of the cells, is detectable within 20–30 min

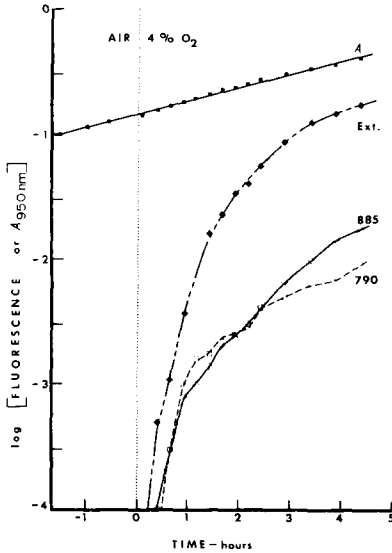


Fig. 2. Growth rate and bacteriochlorophyll production in repigmenting cells of *Rps. spheroides*.  $A_{950\text{ nm}}$ : growth rate; 885 and 790: intensity of fluorescence emission peaks, *in vivo*, at 885 nm and 790 nm, respectively, with 590-nm excitation; Ext.: fluorescence emission intensity at 785 nm of a methanol-acetone extract of the cells, using 595-nm excitation.

after the change of growth conditions and increases rapidly. The significance of the difference in intensity of the fluorescence in the extract and that *in vivo* will be discussed below. After 4–5 h the rate of bacteriochlorophyll synthesis parallels the growth rate of the culture. Comparison with the fluorescence intensity of a known concentration of bacteriochlorophyll indicated that after 4.3 h of growth, the bacteriochlorophyll content was  $0.1 \mu\text{g/ml}$  of culture. Fig. 2 also shows that the fluorescence peak at 790 nm *in vivo* appears simultaneously with that at 885 nm. Initially this fluorescence increases at the same rate, but slows down after 2.5 h. After longer periods of semi-aerobic growth, or after photosynthetic growth (anaerobic) the 790-nm fluorescence is masked by the 885-nm fluorescence.

The organism used for these studies is a green mutant which has only yellow carotenoids. It has absorption maxima at 377, 590, 800 and 850–870 nm due to bacteriochlorophyll and carotenoid peaks at 425, 456 and 487 nm. In the excitation spectrum for the fluorescence emission at 885 nm *in vivo* (Figs. 3 and 4) the same features are seen but are somewhat distorted since only 40–50 % of the energy absorbed by the carotenoids is transferred to the bacteriochlorophyll<sup>18</sup> and also because the

intensity of the quartz-iodine lamp used for excitation decreases sharply in the blue region of the spectrum. The predominant features in the excitation spectrum are a 490-nm peak due to the carotenoids and a 590-nm peak due to the bacteriochlorophyll. The usefulness of these two peaks in a developmental study is seen in Fig. 3. This

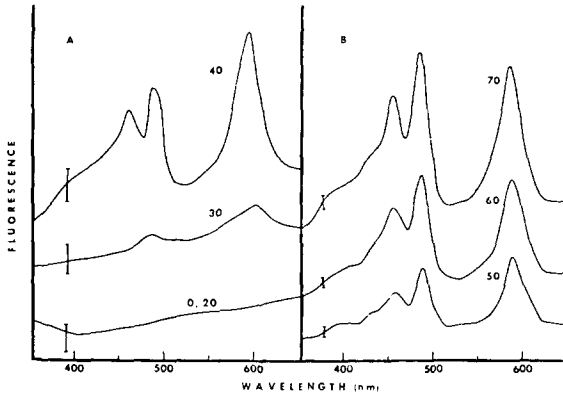


Fig. 3. Fluorescence excitation spectra of bacteriochlorophyll emission at 885 nm in repigmenting cells of *Rps. spheroides*, at various times (min), indicated in the figures, after transfer to semi-aerobic conditions. The intensity scale for A is  $0.3 \times$  that for B and the base lines of subsequent spectra have been displaced upward for clarity. The vertical bars represent the relative noise level of the photomultiplier tube.

shows the increase in the photosynthetic pigment and the energy transfer from the carotenoids to the bacteriochlorophyll during the first 70 min of semi-aerobic growth. As already seen, the bacteriochlorophyll fluorescence can first be detected about 30 min after transfer to semi-aerobic conditions and increases rapidly. Even at this time the contribution from the carotenoids is evident by the appearance of the 490-nm peak. Thus, in the earliest stages of repigmentation the carotenoids are built into the photosynthetic apparatus simultaneously with the bacteriochlorophyll. It is interesting to note that at first the bacteriochlorophyll peak at 590 nm is more intense than

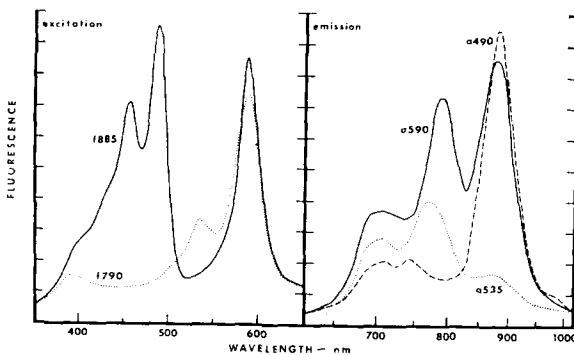


Fig. 4. Fluorescence excitation and emission spectra of cells of *Rps. spheroides* 100 min after transfer to 4% oxygen. 1790 and 1885: excitation spectra for fluorescence emission at 790 and 885 nm, respectively; 490, 535 and 590: emission spectra due to exciting light of 490, 535 and 590 nm, respectively.

the carotenoid peak at 490 nm but that later the carotenoids become more predominant. We will return to this point below.

The fluorescence excitation and emission spectra of whole cells 100 min after transfer from aerobic growth to 4% oxygen are shown in Fig. 4. These enable us to identify the components responsible for the various fluorescence bands and indicate some of their interactions. As in Fig. 3 the excitation spectrum for the 885-nm fluorescence shows both the 490-nm carotenoid and 590-nm bacteriochlorophyll absorption peaks. The emission spectrum using 590-nm excitation shows the emission peaks at both 790 and 885 nm. The emission spectrum using 490-nm excitation shows a predominant emission at 885 nm. In agreement with this, the excitation spectrum of the 790-nm fluorescence shows no contribution from the carotenoids but does show an excitation maximum at 590 nm. Bacteriochlorophyll in methanol-acetone solution has an excitation maximum at 595 nm and an emission maximum at 785 nm. Therefore these data strongly suggest that the 790-nm fluorescence is due to a form of bacteriochlorophyll which has not been bound into the membranes and thus cannot receive excitation energy from the carotenoids. This will be considered further in DISCUSSION.

In addition to the maximum at 590 nm seen in the excitation spectrum for the 790-nm fluorescence, there is a minor peak at approx. 535 nm. However, excitation with 535-nm light results in an emission maximum at 770 nm, rather than 790 nm. These wavelengths (535 nm, 770 nm) are characteristic of bacteriopheophytin<sup>19</sup> and therefore, this result indicates that a form of bacteriopheophytin is also present to a small degree in the intact cells, and that it can be differentiated from the material responsible for the fluorescence at 790 nm.

In order to determine whether this free bacteriochlorophyll and bacteriopheophytin might be artifacts of the semi-aerobic growth conditions, similar spectra were obtained from a young, actively growing, photosynthetic culture. These spectra are presented in Fig. 5. While the emission spectrum from 590-nm excitation shows a small 790-nm emission peak, the spectrum from 490-nm excitation gives no indication of significant emission at 790 nm, and both spectra show the normal emission of bac-

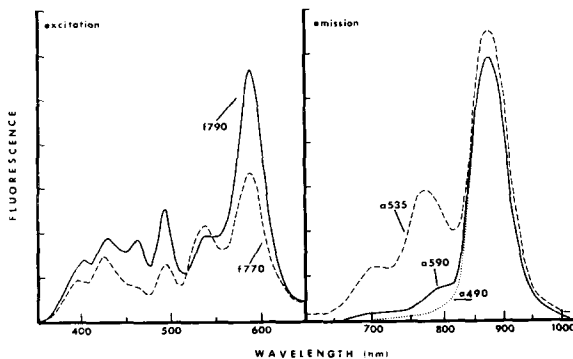


Fig. 5. Fluorescence excitation and emission spectra of cells from a young, actively growing, photosynthetic culture of *Rps. spheroides*.  $\lambda_{770}$  and  $\lambda_{790}$ : excitation spectra for fluorescence emission at 770 and 790 nm, respectively;  $\lambda_{490}$ ,  $\lambda_{535}$  and  $\lambda_{590}$ : emission spectra due to exciting light of 490, 535 and 590 nm, respectively. The intensity scale for the emission spectra using 490-nm and 590-nm excitation is  $10\times$  that employed for the others.

teriochlorophyll at 885 nm. Excitation at 535 nm results in a distinct 770-nm emission, indicating the presence of a bacteriopheophytin-like pigment in these cells as well. The excitation spectra are consistent with these data: the emission at 790 nm is excited predominantly by light at 590 nm, and the emission at 770 nm is more effectively excited by 535-nm light. The small contribution of the carotenoids to the excitation of the 790-nm and 770-nm fluorescence in these photosynthetically grown cells is probably not caused by transfer to the small amount of free bacteriochlorophyll or bacteriopheophytin present but rather is due to the presence of the short wavelength tail of the 885-nm emission from the bound bacteriochlorophyll, which is excited by energy transferred from the carotenoids. Similarly, the emission at 885 nm due to the 535-nm excitation is most reasonably explained as a result of a small carotenoid or bacteriochlorophyll absorption at 535 nm.

We have used sodium dithionite to obtain an indication of the interaction between the pigment system and electron transport pathways and, by extrapolation, to obtain a measure of the photosynthetic capacity of the cells. CLAYTON<sup>20</sup> has reported that the addition of dithionite causes about a two-fold increase of bacteriochlorophyll fluorescence in photosynthetic cells; a nonphotosynthetic, pigmented mutant does not show this effect. PARSON<sup>21</sup> has shown that dithionite prevents photooxidation of the reaction-center pigment, P870. Thus, the excitation energy normally utilized for photochemistry is lost as excess fluorescence. We have observed that the 885-nm fluorescence excited by either 490-nm or 590-nm light increases on addition

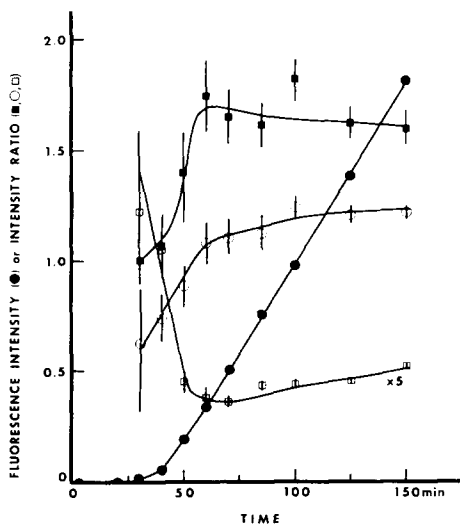


Fig. 6. The increase in extractable bacteriochlorophyll fluorescence intensity at 875 nm (arbitrary units) (●), in the dithionite effect (the ratio of the fluorescence intensity *in vivo* at 885 nm after dithionite addition to that before addition, using 590-nm excitation) (■), and in the relative energy transferred from carotenoids to bacteriochlorophyll (the ratio of the fluorescence intensity *in vivo* at 885 nm due to excitation with 490-nm light to that due to excitation with 590-nm light) (○) and the decrease in the relative fluorescence yield *in vivo* (the ratio of the fluorescence intensity at 885 nm *in vivo* to that in a methanol-acetone extract at 875 nm, excited by 590-nm light and 595-nm light, respectively) (□) during repigmentation in *Rps. spheroides*. The vertical bars indicate the uncertainty in the values, as determined from the noise level of the photomultiplier tube.

of dithionite but that the 790-nm fluorescence is unchanged. Thus, the dithionite effect is manifested only in those pigments directly coupled to the photochemical apparatus. This supports the assumption that dithionite does not affect the pigments directly but acts on the electron-transport system.

We have obtained a measure of this dithionite effect by taking the ratio of the fluorescence intensity emitted at 885 nm, due to excitation by 590-nm light, after dithionite addition to that before addition. This has been used to compare the state of the pigment system at different stages of development. Two additional parameters which we have used to characterize the repigmenting cells are (1) the ratio of carotenoid to bacteriochlorophyll effectiveness in exciting the 885-nm bacteriochlorophyll emission and (2) the relative fluorescence yield *in vivo*. In Fig. 6, we show how these parameters change during repigmentation. In the first hour, during which only 0.1–0.2 % of the total amount of bacteriochlorophyll present in fully pigmented cells grown at moderate light intensity has been synthesized, the sensitivity to dithionite increases at the same time that the relative contribution of energy transferred from the carotenoids increases and the fluorescence yield of the main bacteriochlorophyll component decreases. The plateau values of all three of the parameters, which are attained within the first 60–70 min of pigment synthesis, are virtually identical with the values observed in mature, photosynthetically grown cells.

#### DISCUSSION

The repigmentation process observed in our experiments is similar to that reported by others for both *Rhodospirillum rubrum* and *Rps. spheroides*<sup>6,7,14,15</sup>. There are, however, some important differences. Due to initial aerobic growth in the light rather than in the dark, we have achieved a greater degree of bleaching than would appear to be the case in the other reports, with the possible exception of the work of GORCHEIN *et al.*<sup>14</sup>. These latter workers carried out the initial growth in pure oxygen rather than air in order to obtain depigmented cells. COHEN-BAZIRE *et al.*<sup>6</sup> suggested that pigment is not destroyed during aerobic growth but simply diluted out as a result of cell division in the absence of pigment synthesis. However, we have observed (unpublished experiments) that after a certain degree of dilution has been achieved, some additional bleaching or destruction of the pigment does occur in the light. We are unable to detect any bacteriochlorophyll fluorescence in our bleached cells, which indicates that there is initially less than 0.3 ng of bacteriochlorophyll per ml of culture as compared to 0.1  $\mu\text{g/ml}$  after 4 h of semi-aerobic growth. The rapidity with which new pigment becomes detectable is remarkable. We detected pigment 30 min after transfer. In contrast, GORCHEIN *et al.*<sup>14</sup> reported a measurable increase in bacteriochlorophyll "within 2 h of changing the conditions" in the case of *Rps. spheroides*, and similar lags have been reported by LASCELLES<sup>7</sup>. We feel that the major factor in the difference between our results and those of these other investigators is the increased sensitivity with which we can detect bacteriochlorophyll with our fluorescence technique. The behavior of the different strains of bacteria that are used could be another source of the difference. BIEDERMANN *et al.*<sup>15</sup> do report similar induction periods in the case of *Rhodospirillum rubrum*, but from their Fig. 3, it appears that their cells contain 3 ng of bacteriochlorophyll per ml of culture at the time of transfer to semi-aerobic conditions. Thus, the cells employed by these workers contain at least an



order of magnitude more bacteriochlorophyll after bleaching than those we have used.

We suggested above that the prominent fluorescence emission at 790 nm during the early stages of development is due to a form of bacteriochlorophyll which is free, *i.e.* it has not yet been bound into the membranes. It is entirely reasonable that this material is bacteriochlorophyllide, which would be expected to have spectral characteristics almost identical to bacteriochlorophyll<sup>22</sup>. This material is relatively unstable, and if whole cells are stored at 4° for several hours, the emission peak shifts from 790 to 770 nm. As we have reported elsewhere<sup>17</sup> there is also some material excreted into the medium which fluoresces at about 770 nm. In additional experiments (unpublished) we have found that the excitation maximum for this 770-nm emission occurs at 535 nm. This material is probably a form of bacteriopheophytin or bacteriopheophorbide, and we suggest that the "unbound" bacteriochlorophyll is the final product of the bacteriochlorophyll biosynthetic pathway and is in a "pool" from which it is then incorporated into the membrane. The bacteriopheophytin-like pigment formed *in vivo* and excreted into the medium is then the result of the degradation of this unbound bacteriochlorophyll. This indicates that incorporation into the membrane stabilizes the bacteriochlorophyll against degradation. The suggestion that the 790-nm fluorescence is due to the unphytylated bacteriochlorophyll is based on the relatively high water solubility of the form of bacteriopheophytin which is excreted, and it seems entirely reasonable that the last step in the incorporation into the membrane involves the attachment of the phytol.

In unpublished experiments on pigment development in a carotenoidless mutant of *Rps. spheroides* (strain R-26; see ref. 23), we have observed approximately a 10-fold greater production of excreted and intracellular pigments, which fluoresce at 770–790 nm, during semi-aerobic growth than in the strain used for the studies reported here. A young, photosynthetically grown (anaerobic) culture of this mutant also shows a pronounced 790-nm fluorescence emission peak, due to the unbound form of bacteriochlorophyll, about five times as intense as in our standard strain. These results indicate that there is a slower rate of incorporation of the "free" form into the membrane due to the absence of carotenoids. This, along with the evidence for the simultaneous incorporation of carotenoids presented in this paper, suggests that the carotenoids may possibly have an integral role in membrane structure and synthesis.

A major aspect of this study has been to attempt to correlate membrane synthesis and pigment interaction, as measured by the spectral changes, with the onset of photochemical activity. As discussed above, we have employed sodium dithionite to obtain an indication of the amount of interaction between the pigment and electron-transport systems in the membrane. While the amount of fluorescence increase upon dithionite addition does not give a direct measure of the total photosynthetic capability of the cells, particularly their ability to carry out photophosphorylation or CO<sub>2</sub> fixation, it does offer two distinct advantages. First, it retains the characteristic speed and sensitivity of the fluorescence technique, and, second, it measures the activity which is of major interest in this study, that of the reaction center *per se*. The fact that dithionite addition causes a fluorescence increase from the beginning of detectable pigment synthesis suggests that the reaction center is formed along with the bulk bacteriochlorophyll and interacts immediately with the pigment and electron-transport systems, as opposed to being synthesized later and incorporated in a final step which confers activity.

VREDENBURG AND DUYSSENS<sup>24</sup> and more recently CLAYTON<sup>25</sup> have shown that the bacteriochlorophyll fluorescence yield *in vivo* increases upon illumination in such a way as to parallel the bleaching of the reaction center pigment in aerobic suspensions of whole cells of *Rhodospirillum rubrum* and *Rps. spheroides*. Thus an alternative to the use of the dithionite effect as an index of trapping would be the use of time-variation of fluorescence yield during excitation. This should indicate the presence of reaction centers that become saturated after a given time. The effect should be dependent upon the illumination intensity, being present at high light intensity but not at low ones. This would in turn result in the steady-state yield of fluorescence being dependent upon the illumination intensity. Measurements of this type, however, require instrumentation capable of rapid (msec) time resolution which we do not currently possess.

The increase in the relative amount of energy transferred to the bulk bacteriochlorophyll from the carotenoids, as measured by the increasing carotenoid-bacteriochlorophyll ratio in Figs. 3 and 6, is most probably due to a decrease in the intermolecular distance between the carotenoid and bacteriochlorophyll molecules as the pigments are incorporated into the membrane. Similarly, the decrease at the same time in the relative fluorescence yield *in vivo* could be due either to concentration quenching (see ref. 26, for example) or to photochemical utilization, or both, facilitated by increased intermolecular energy transfer as the concentration increases. This decreased yield is the cause of the difference in the fluorescence intensities at 885 nm *in vivo* and at 785 nm in the extract seen in Fig. 2. The similar behavior of the dithionite effect and the fluorescence yield suggests that photochemical utilization is a major cause of the decrease in yield. In addition, the carotenoid-bacteriochlorophyll ratio also attains a relatively constant value at the time that the dithionite effect becomes maximal. This simultaneity strongly indicates that the entire photosynthetic pigment system is constructed coordinately as a functional unit from the onset of pigment synthesis. Thus, when cells contain only 0.1–0.2% of the total amount of bacteriochlorophyll present in fully pigmented cells grown at moderate light intensity, the reaction centers are as functional and efficient as those in the latter.

BUTLER<sup>27,28</sup> and GOEDHEER<sup>29</sup> have made spectral studies similar to ours in greening bean leaves which show that energy transfer from carotenoids and quenching of chlorophyll fluorescence, *in vivo*, occur within 4–5 h after chlorophyll synthesis is first induced. Neither of these workers made direct correlations with the development of photosynthetic activity in their material, although BUTLER<sup>28</sup> attributed the decrease in fluorescence yield to the development of activity and concluded both photoreaction systems “are active as soon as their pigments appear”. Our data discussed above tend to verify the assumption of BUTLER that the decrease in yield is due to the onset of activity and not just to concentration quenching in the membranes. OHAD *et al.*<sup>30</sup> conducted an electron microscopic study of the development of chloroplast membranes in a mutant of *Chlamydomonas*. They also determined the activity of various photosynthetic enzymes and reactions and measured the amount of chlorophyll present at various stages. Their conclusions were consistent with those of BUTLER<sup>28</sup> on bean leaves, in that the assembly of the total photosynthetic apparatus occurs as a single step process. Our studies on the analogous process in the photosynthetic bacteria, with a more sensitive measure of the changes occurring among the pigment molecules in the developing membrane, point out the striking similarity in

the process of photosynthetic membrane biosynthesis in these different organisms.

We have no information at present about the other components of the electron-transport system during the developmental sequence, and do not know what changes, if any, occur when pigment synthesis is induced, what their rate of synthesis is, or how they are integrated into the apparatus in order to attain photosynthetic activity. A developmental study of cytochromes as well as morphological changes would be desirable during the early stages in pigment and membrane synthesis where the pigment density is undoubtedly low and the membrane network is probably only beginning to be formed. Such studies would offer additional insight to interactions at the reaction center and provide information on the mechanism of thylakoid biogenesis.

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