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TOWARD THE ISOLATION OF A PHOTOCHEMICAL REACTION CENTER IN *RHODOPSEUDOMONAS SPHEROIDES*

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SUMMARY

Chromatophores of *Rhodopseudomonas spheroides* show reversible light-induced absorbancy changes corresponding to the oxidation of P870 (a pigment resembling bacteriochlorophyll), the oxidation of one or more cytochromes, and the reduction of ubiquinone. The light-reacting P870 is accompanied by a much larger amount of "light-harvesting" bacteriochlorophyll.

Upon prolonged anaerobic incubation in the light, most of the bacteriochlorophyll in cells of blue-green mutant *R. spheroides* is converted *in situ* to bacteriopheophytin. In chromatophores from such cells the light-reactions of P870, cytochrome, and ubiquinone have the same size and character as in chromatophores from "fresh" cells. Under suitable conditions P870 can be isolated as a spectrophotometric entity; *i.e.*, as an absorption band at 870 mμ that is bleached completely and reversibly by light.

Excitation spectra for fluorescence of bacteriochlorophyll (at 900 mμ) and bacteriopheophytin (at 780 mμ), and for generation of the light-reactions of P870 and cytochrome, show the occurrence of energy transfer from bacteriopheophytin to bacteriochlorophyll and from bacteriopheophytin to P870, as well as from bacteriochlorophyll to P870. Kinetics of the 900-mμ fluorescence indicate that bacteriochlorophyll is fluorescent but P870 is not

Extraction and analysis of pigments in "pheophytinized" chromatophores indicates that P870 is simply bacteriochlorophyll in a specialized environment.

These experiments define a photochemical reaction center built around P870 as a terminal acceptor of excitation energy. The reaction center is served by light-harvesting molecules; these can be either bacteriochlorophyll or bacteriopheophytin.

INTRODUCTION

Current theories of photosynthesis[1,2] stipulate the operation of photosynthetic units: sets of chlorophyll molecules that harvest the energy of light quanta and direct this energy to photochemical reaction centers. The reaction centers, receiving energy, effect a separation of oxidizing and reducing entities and thus provide starting points for the electron transfer reactions of photosynthesis.

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin.

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Photosynthetic tissues exhibit various light-induced absorption spectrum changes that reflect reversible photochemical reactions. The character of these changes has implicated certain molecular species as constituents of photochemical reaction centers In green plants and algae a photochemical system appears to be built around P700, a pigment having an absorption maximum at about 705 mμ[3,4]. P700 may be simply chlorophyll *a* in a specialized environment[5]. Also implicated in this system are one or more cytochromes[6] and (provisionally) plastoquinone[7]. Reactions of these substances have been observed spectrophotometrically; the kinetics indicate that an electron is transferred from P700 to plastoquinone, after which the P700 regains an electron from cytochrome[4].

An analogous system has been delineated[8-13] in the chromatophores of photosynthetic bacteria, with BChl in place of chlorophyll *a*. The analogue of P700 is a pigment whose bleaching is maximal at 870–890 mμ, depending on the species of bacterium. This pigment has been termed $BChl_2$ (ref. 12) or P890 (ref. 10). It is probably BChl in a specialized environment (see later), but until this point is settled rigorously the suggestive term "$BChl_2$" will be abandoned. The term P890 will be used for *Chromatium* and *Rhodospirillum rubrum*, and P870 for *R. spheroides*, corresponding to "bleaching maxima" at 890 and 870 mμ, respectively.

The light-reaction of P870 is mimicked by chemical oxidation[12,14,15] and the restoration of P870 after a flash of light is accelerated in a reducing environment (R. K. CLAYTON, unpublished). The reaction of P870 is therefore identified provisionally as an oxidation.

If P870 has the same molar extinction coefficient as BChl, the molecular ratio of P870 to BChl in *R. spheroides* chromatophores is about 1:30 (ref. 13). In green plant tissues the ratio of P700 to chlorophyll *a* is about 1:400. Thus the study of P870 or P890 in photosynthetic bacteria, and of P700 in green plants, has been complicated by the presence of much larger amounts of BChl or chlorophyll. Methods have now been developed for removing, from chromatophores of *R. spheroides*, most of the "light-harvesting" BChl without impairing the observable light-reactions. In these treated chromatophores P870 can be isolated as a spectrophotometric entity and some of its properties can be determined. Evidence will be presented here that P870 is BChl in a specialized environment.

MATERIALS AND METHODS

Materials

R. spheroides, carotenoidless (blue-green) mutant strain 2.4.1/CCl/R-26, was cultivated anaerobically in the light as described earlier[16,17]. Chromatophores were harvested from sonic extracts of this organism by centrifugation for 90 min at 100000 × *g*. The chromatophores were washed twice and suspended in distilled water for experimental use. The principal infrared absorption maximum of BChl in such chromatophores was at 870 mμ.

Special treatments

When a mature culture of blue-green mutant *R. spheroides* was exposed to light under anaerobic conditions, the BChl in the cells was gradually converted to BPh. About 10% of the BPh was excreted by the cells, appearing in the culture medium as

a microcrystalline suspension. The conversion was nearly complete after 20-days exposure, at room temperature, to the light from a 100-W tungsten lamp located 10 in from the culture. Such "pheophytinized" cells yielded chromatophores in which most of the BChl was replaced by BPh, but in which the reversible light-induced absorbancy changes (including the bleaching of P870) were essentially the same as in chromatophores from "fresh" cells. The conversion of BChl to BPh did not occur in cells of wild type *R. spheroides*.

BChl could also be destroyed in chromatophores of blue-green mutant *R. spheroides* through exposure to the non-ionic detergent Triton X-100. Chromatophores, suspended in distilled water saturated with air and containing 1% Triton X-100, gradually lost their BChl when illuminated. After several hours of moderate illumination much of the BChl was eliminated, leaving no obvious product of its degradation. In these detergent-treated chromatophores (or chromatophore fragments) the light-induced absorbancy changes were practically the same as in untreated chromatophores.

The combination of pheophytinization and detergent treatment gave preparations in which the only material absorbing at 870 mμ was P870; *i.e.*, in which the reversible light-induced bleaching at this wavelength was complete.

Characterization and assay of pigments

BChl and BPh were extracted from chromatophores with a mixture of acetone–methanol (7:2, v/v). Water was removed from the extract by adding Na_2SO_4; the

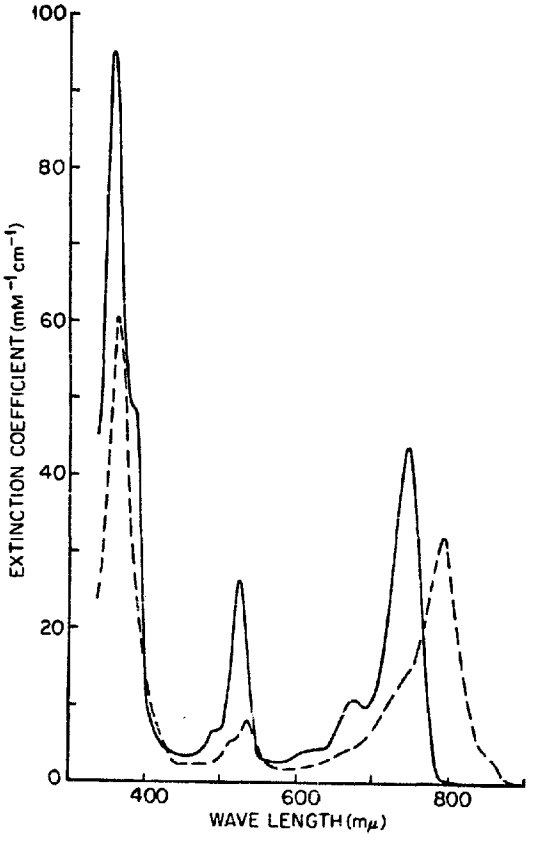

Fig. 1. Absorption spectra of BPh from chromatophores of blue-green mutant *R. spheroides*. Solid curve, pigment in acetone–methanol (7:2, v/v). Dashed curve, aggregated pigment suspended in H_2O.

extract could then be evaporated to dryness and the pigments dissolved in other solvents. BChl and BPh were separated chromatographically on powdered sugar, with petroleum ether containing 1% pyridine as the liquid phase. The pigments were identified by their absorption spectra in ether, in chloroform, and in methanol (see ref. 18). The BPh extracted from chromatophores had the same absorption spectrum as BPh prepared from BChl by the method of FRENCH[19].

Absorption spectra were measured for equal concentrations of BChl in various media: in dry ether, in acetone–methanol (7:2, v/v), and in aqueous chromatophore suspensions (*in vivo*). In ether the extinction coefficient[16-20] of BChl at 770 mμ is 94 mM^{-1} cm^{-1}. Comparison yielded extinction coefficients of 75 mM^{-1} cm^{-1} at 770 mμ in acetone–methanol, and 130 mM^{-1} cm^{-1} at 870 mμ *in vivo* (for blue-green mutant *R. spheroides*). The last two figures supersede the values of 41 and 73, respectively, used in an earlier report[13]. The earlier values had been computed from data reported by COHEN-BAZIRE *et al.*[21].

Absorption spectra of BPh in H_2O (suspension of crystals) and in acetone–methanol (7:2, v/v) are shown in Fig. 1. The values of extinction coefficients are based on a comparison with the spectrum of BPh in chloroform; in this solvent the extinction coefficient[19] at 530 mμ is 25 mM^{-1} cm^{-1}. The spectrum of aggregated BPh in H_2O appeared to be the same as that of BPh retained in chromatophores (*cf.* Figs. 1 and 4a). BPh *in vivo* could therefore be estimated from absorption spectra of cells or chromatophores; the validity of such estimates was confirmed by assays based on extraction and chromatographic purification of the BPh.

Optical measurements

The technique of measuring light-induced absorbancy changes in chromatophores has been described elsewhere[8,12]. The method employed a beam of exciting light and a measuring beam. The detector for the measuring beam was shielded from the exciting light through appropriate placement of color filters. This technique was used with Beckman, Zeiss and Cary spectrophotometers. Monochromatic exciting light was obtained with a Perkin-Elmer model 83 monochromator; the intensity of this light was determined with a calibrated Eppley thermopile. Excitation spectra for the generation of absorbancy changes could then be measured.

Fluorescence spectra and excitation spectra for fluorescence were measured with a Leiss monochromator, its output energy again determined with a calibrated Eppley

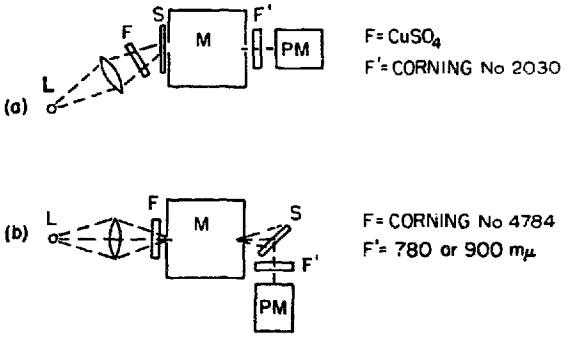

Fig. 2. Apparatus for measuring fluorescence and fluorescence excitation spectra (schematic). (a) Arrangement for fluorescence spectra, (b) arrangement for fluorescence excitation spectra. M = Monochromator, PM = photomultiplier, S = quartz sample chamber, L = tungsten lamp. F and F′ are filters, the 780- and 900-mμ types being interference filters.

thermopile. In obtaining spectra, an RCA 7102 (infrared-sensitive) photomultiplier was used as detector. The arrangements for determining fluorescence spectra and fluorescence excitation spectra are shown schematically in Fig. 2.

LIGHT-INDUCED ABSORBANCY CHANGES AND THE NATURE OF P870

Absorption spectra of chromatophores from blue-green mutant *R. spheroides*, either fresh, pheophytinized, or treated with Triton X-100, are to be found in Figs. 3 and 4. Fresh chromatophores (Fig. 3a) showed the characteristic BChl band at 870 mμ. Pheophytinized chromatophores (Fig. 4a) showed, instead, a major band at about 800 mμ due to BPh. In the presence of Triton X-100 (Fig. 4b) this peak was shifted to 750 mμ; the aggregated BPh excreted by *R. spheroides* cells behaved in the same way. This shift could be expected if the detergent converted aggregated BPh to a unimolecular state. In fresh chromatophores that had been treated with Triton X-100 (Fig. 3b), the peak at 870 mμ was reduced about 20-fold and a band at 805 mμ became predominant. To account for the amount of BChl that could be extracted from such chromatophores, this 805-mμ band should be ascribed mainly to BChl rather than to BPh.

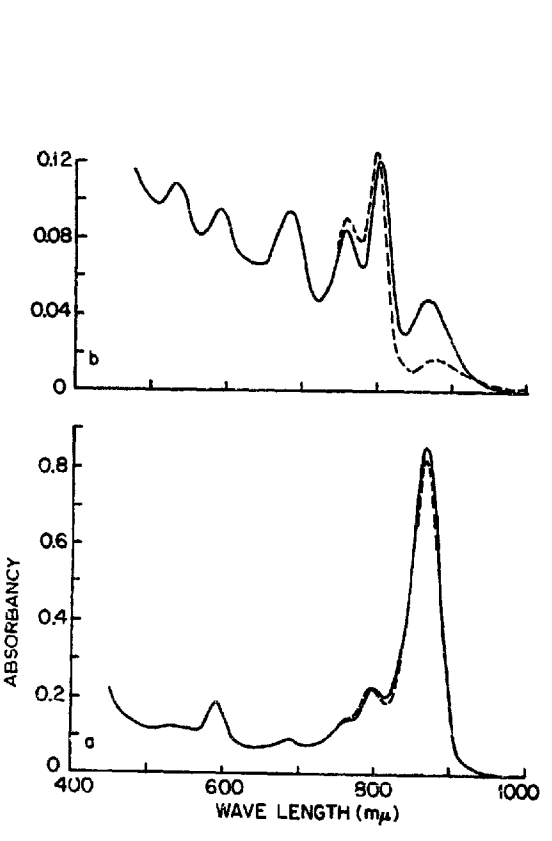

Fig. 3. Absorption spectra of chromatophores from freshly grown blue-green mutant *R. spheroides* cells. a, Untreated; b, treated with 1% Triton X-100 (see text for details). Solid curves: no exciting light. Dashed curves: chromatophores exposed to blue-violet exciting light during measurement of absorbancy.

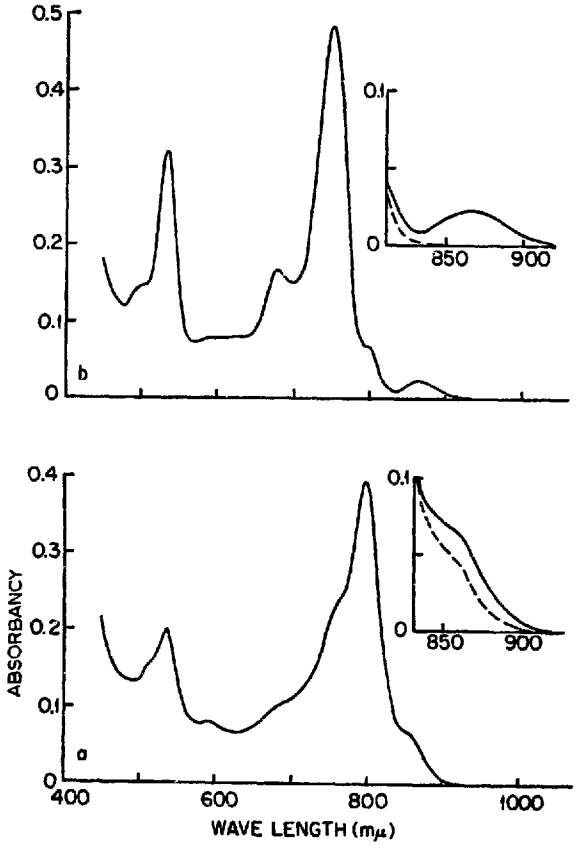

Fig. 4. Same as Fig. 3, but for chromatophores from pheophytinized *R. spheroides* cells. a, Untreated; b, treated with Triton X-100. Solid curves, exciting light off. Dashed curves, exciting light on.

The spectrum of reversible, light-induced absorbancy changes in chromatophores of blue-green mutant *R. spheroides*, demonstrating the bleaching of P870, is shown in Fig. 5. This figure pertains to chromatophores in which most of the BChl had been converted to BPh (see Fig. 4a). A nearly identical spectrum was obtained with "fresh" chromatophores in which the destruction of BChl was negligible (*cf.* Fig. 1 of ref. 12), and also with chromatophores that had been treated with Triton X-100 (Figs. 3b and 4b). In addition to the features seen in Fig. 5, the following absorbancy changes occurred: (1) changes in the region 240–300 mμ that suggest reduction of ubiquinone; (2) changes that correspond to cytochrome oxidation, observed only in thoroughly dark-adapted preparations; (3) changes corresponding to a slight blue-shift of absorption bands at 375 and 590 mμ, and bleaching at 600 mμ; (4) appearance (in the light) of absorption bands at 435, 710, and 1000–1250 mμ. All of these changes were exhibited by pheophytinized and/or detergent-treated chromatophores, in the same relative proportions as in fresh chromatophores.

Table I shows the effects of pheophytinization and detergent treatment on the amounts of BChl, BPh, and P870 in chromatophores of blue-green mutant *R. spheroides*. Samples 1–4 represent progressively greater degrees of pheophytinization. It can be seen that most of the BChl that became lost could be accounted for as BPh. In these determinations the amount of P870 was judged from the maximal reversible bleaching at 870 mμ, with the assumption that P870 has the same extinction coefficient as BChl *in vivo*. Amounts of BChl and BPh were estimated from absorption

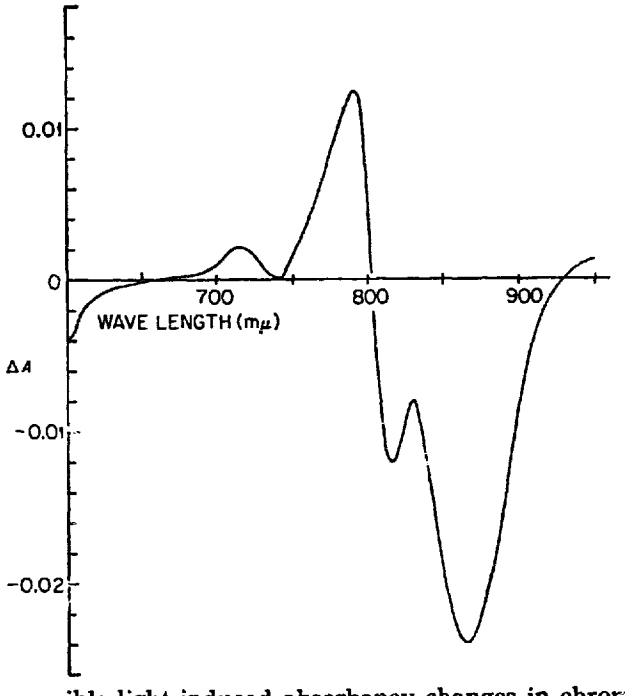

Fig. 5. Spectrum of reversible light-induced absorbancy changes in chromatophores from pheophytinized *R. spheroides* cells. For absorption spectrum see Fig. 4a.

spectra of disrupted cell suspensions. Samples 1 and 3 were analyzed further through extraction and chromatography of the pigments; the amounts of BChl and BPh recovered chromatographically agreed within ± 20% with the values given in Table I.

It can be seen from Fig. 4b that P870 is a substance whose 870-mμ absorption

band becomes entirely lost, reversibly, in the light. We shall now consider whether this substance resembles BChl. If P870 is BChl, it should possess an absorption band at about 590 mμ, roughly one sixth as high as the 870-mμ band. Chromatophores do show a reversible bleaching at 600 mμ, about one sixth as great as the bleaching at 870 mμ (Fig. 5; also Fig. 1 of ref. 12). This already indicates that P870 is BChl, but let

TABLE I

PIGMENTATION OF *R. spheroides* IN SUCCESSIVE STAGES OF PHEOPHYTINIZATION

	Progressive pheophytinization Sample No.				*Sample 2 after treatment with 1% Triton X-100*
	1	*2*	*3*	*4*	
μmoles* BChl**	10.6	9.3	2.5	0.0	0.9
μmoles BPh	2.1	3.6	7.6	9.4	1.2
μmoles P870	0.4	0.4	0.3	0.3	0.3

* Per g of dry cell mass.
** Other than P870.

TABLE II

AMOUNTS OF LIGHT-REACTING PIGMENT (P870), OF NON-REACTING BACTERIOCHLOROPHYLL (B870), AND OF TOTAL BChl IN *R. spheroides* CHROMATOPHORE PREPARATIONS

Sample	*P870 (mμmoles)*	*B870 (mμmoles)*	*BChl (mμmoles)**
A	0.62	0.04	0.88
B	0.82	0.15	0.80
C	1.28	1.12	1.82

* BChl was determined from the absorption band at 590 mμ *in vivo*.

us go further. If BChl is the only substance, abundant in chromatophores, that has an absorption maximum at 590 mμ, two possibilities can be listed. (1) P870 is BChl, and the height of the 590-mμ band can be correlated with the total amount of BChl. This total includes P870 and the "ordinary" BChl that does not show a light-reaction. "Ordinary" BChl will be termed B870, after VREDENBERG AND DUYSENS[10], in the present context. (2) P870 is not BChl, and the height of the 590-mμ band corresponds to the amount of B870 alone.

The amount of P870 is given by the extent of bleaching at 870-mμ. The sum of P870 and B870 is given by that part of the $A_{870\,m\mu}$ that is not attributable to BPh. (The A attributable to BPh can be estimated with the help of Fig. 1.) Using these criteria, the amounts of P870 and B870 have been computed, together with the amount of BChl that corresponds to the absorption band at 590 mμ, for three different preparations of pheophytinized chromatophores. Results are shown in Table II. The amount of BChl computed from the 590-mμ band is clearly greater than the amount of B870, and is comparable to the sum of P870 and B870. P870 thus appears to be a substance having an absorption spectrum similar to that of BChl in the neighborhood of 590 mμ as well as at 870 mμ.

The foregoing computations are of questionable accuracy for several reasons. Had

fresh chromatophores been used, the amount of P870 would have been a small fraction of the amount of BChl. In that case the data would not have afforded a decision as to whether P870 is a form of BChl. But with pheophytinized chromatophores it was necessary to analyze absorption spectra wherein the features pertaining to P870 and B870 were overshadowed by the spectrum of BPh (see Fig. 4a). Here the amount of BChl could not be determined accurately. It was therefore undertaken to extract the pigments and remove the BPh chromatographically. The amount of BChl found in the chromatographed extract could then be compared with the amounts of P870 and B870 present before extraction. Results are shown in Table III; included in the table are data for fresh chromatophores. The latter data indicate the per cent recovery of BChl that was attained in the analysis. The four examples for pheophytinized chromatophores pertain to separate analyses of aliquots of a single sample. Again it appears that P870 is BChl, since the amount of BChl recovered from pheophytinized chromatophores is greater than the amount of B870 originally in them.

TABLE III

AMOUNTS OF LIGHT-REACTING PIGMENT (P870), OF NON-REACTING BACTERIOCHLOROPHYLL (B870), AND OF TOTAL BChl IN *R. spheroides* CHROMATOPHORE PREPARATIONS

Sample	*P870 (mμmoles)*	*B870 (mμmoles)*	*BChl (mμmoles)**
Pheophytinized chromatophores	2.8	3.6	4.7
	2.8	3.6	8.6
	2.8	3.6	5.3
	2.8	3.6	4.3
Fresh chromatophores	4.0	78	75

* BChl was determined as pigment extracted with acetone-methanol and purified by chromatography on sucrose.

P870 appears to be a specialized form of BChl that can engage in a reversible photochemical reaction. The specialization may have to do with an association with molecules such as ubiquinone and cytochrome. P870 is able to withstand conditions that cause alteration of the major component of BChl in blue-green mutant *R. spheroides*. In chromatophores that contain colored carotenoids (*e.g.*, those of wild type *R. spheroides*), the major BChl component, as well as the P870 fraction, withstands the conditions leading to pheophytinization. Thus the conversion of BChl to BPh *in vivo* depends strongly on associations between BChl and other molecules.

ENERGY TRANSFER AND FLUORESCENCE

Further information regarding the nature of P870 and its relationship to other pigments could be gleaned from measurements of fluorescence and from excitation spectra that signify intermolecular energy transfer. These measurements will now be described.

Partially pheophytinized chromatophores of blue-green mutant *R. spheroides* show absorption bands at 535, 760, and 800 mμ due to BPh, and at 590 and 870 mμ due

to BChl. Absorption and fluorescence spectra of such chromatophores are shown in Fig. 6. The fluorescence band at 890 mμ is due to BChl; the band at 770 mμ and the shoulder at 815 mμ are due to BPh. Evidently the component absorbing at 760 mμ is more strongly fluorescent than the 800-mμ component. This is consistent with the view that the 760-mμ band represents unimolecular BPh whereas the 800-mμ band is a property of aggregated BPh. Aggregation enhances the likelihood of radiationless de-excitations and thus suppresses fluorescence.

Kinetics of the fluorescence at 780 mμ (BPh) and at 900 mμ (BChl) are also shown in Fig. 6. The intensity of the fluorescence is seen to increase while the exciting light is on; this effect is more pronounced for the BChl fluorescence. A similar rise in BChl fluorescence has been studied in detail by VREDENBERG AND DUYSENS[10], who showed that in *Rhsp. rubrum* the rise in fluorescence intensity could be correlated quantitatively with the bleaching of P890 that occurred while the exciting light was on. VREDENBERG AND DUYSENS reasoned that as long as P890 was intact, it acted as an efficient sink for excitation energy in the BChl system. When P890 became bleached

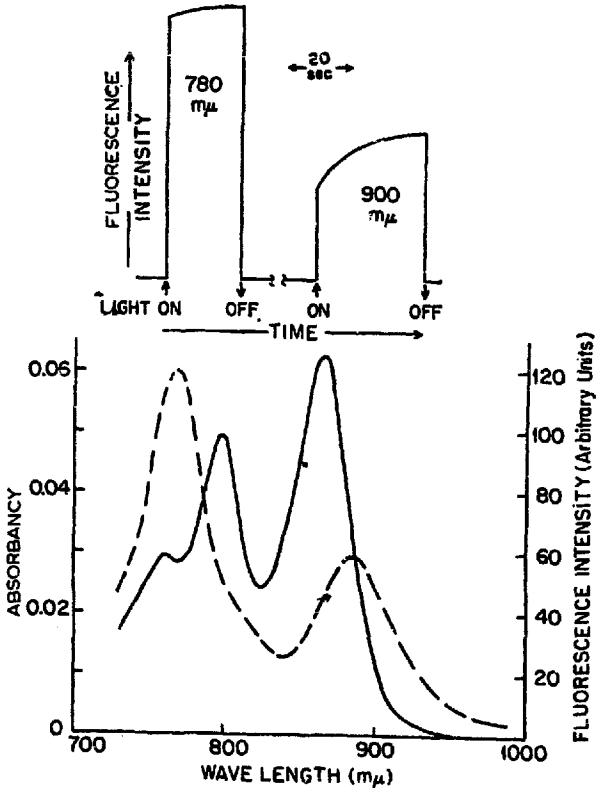

Fig. 6. Absorption and fluorescence spectra of chromatophores from partially pheophytinized *R. spheroides* cells. Solid curve, absorption. Dashed curve, fluorescence. The kinetics of the fluorescence intensity, under constant exciting illumination, are shown above the spectra.

it lost its ability to trap energy. Other pathways of energy dissipation, including fluorescence, were then favored. The preliminary results obtained with *R. spheroides* (Fig. 6) are consistent with the findings of VREDENBERG AND DUYSENS. The time-course of 780-mμ fluorescence showed a slight rise (at 900 mμ the rise was more pronounced). In terms of the foregoing interpretation, this result suggests that some excitation

energy in BPh could be trapped by P870. The occurrence of such energy transfer will be confirmed when excitation spectra are considered.

In a preparation such as that of Fig. 4a, wherein practically all the BChl had been converted to BPh, the amount of B870 (*i.e.*, of BChl other than P870) was much less than the amount of P870. If P870 itself is fluorescent, the intensity of fluorescence at 900 mμ could have been expected to decline as the P870 became bleached by the exciting light. Actually the very slight fluorescence at 900 mμ, in preparations of this kind, remained constant during excitation. It was concluded that P870 is non-fluorescent. The steady fluorescence at 900 mμ could be attributed to a trace of B870 that was unable to transfer energy to P870. Lack of energy transfer from B870 to P870 could be expected in this case. Both are present in small amounts, probably isolated by the surrounding BPh.

Excitation spectra for the 780-mμ fluorescence of BPh and the 900-mμ fluorescence of BChl are shown in Fig. 7. The preparation was the same as that described in Fig. 6; its absorption spectrum from 450 to 650 mμ is also shown in Fig. 7. It can be seen that the fluorescence of BPh was sensitized mainly by PBh itself and by substances absorbing at 430–450 mμ. The latter are probably products of degradation of BChl. The excitation spectrum of BChl fluorescence shows a major band at 590 mμ

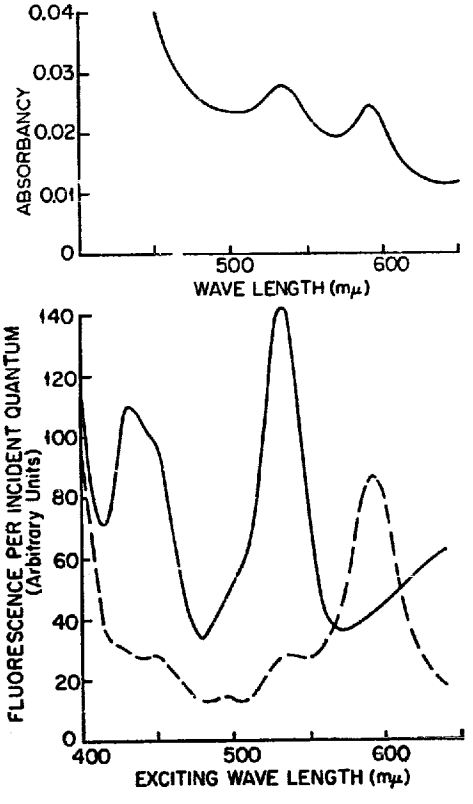

Fig. 7. Absorption spectrum and excitation spectra for fluorescence of *R. spheroides* chromatophores (same preparation as in Fig. 6). Lower solid curve, excitation of 780 mμ (BPh) fluorescence. Dashed curve, excitation of 900 mμ (BChl) fluorescence.

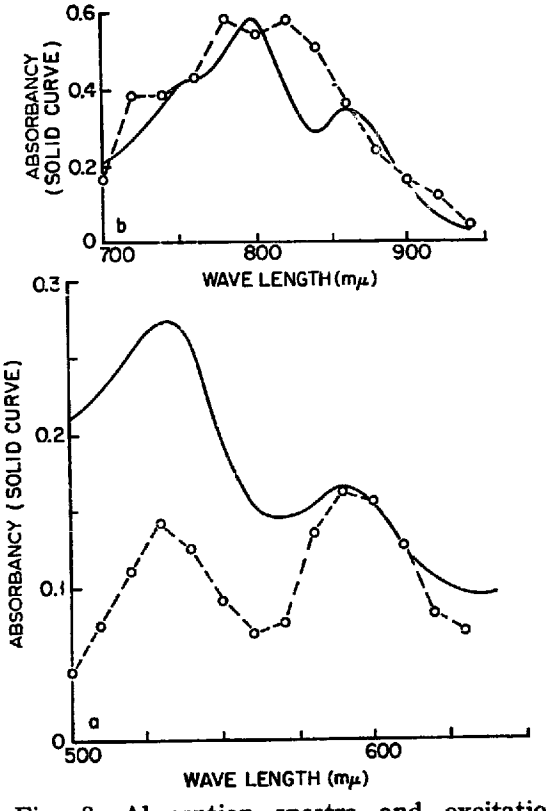

Fig. 8. Absorption spectra and excitation spectra for the generation of light-induced absorbancy changes, in partially pheophytinized *R. spheroides* cells and chromatophores. a, Chromatophores; excitation of $\Delta A_{870\ m\mu}$ (bleaching of P870). b, Cells; excitation of $\Delta A_{420\ m\mu}$ (oxidation of cytochrome). The solid curves are absorption spectra; the dashed curves are excitation spectra. For the latter, the ordinate represents ΔA per unit of exciting light intensity (intensity computed in quanta/cm^2 sec).

(BChl) and a lesser one at 535 mμ (BPh). Excitation spectra for BChl fluorescence in fresh chromatophores (containing little BPh) lacked the 535-mμ band. The relative heights of the bands at 535 and 590 mμ, for fluorescence excitation and for absorption, showed that in the preparation of Fig. 7 energy was transferred from BPh to BChl with a quantum efficiency of about 25%.

Excitation spectra were measured, using a different preparation, for the elicitation of light-induced absorbancy changes. Two excitation spectra were obtained: one in the visible region, for the sensitization of P870 bleaching in chromatophores, and one in the infrared for the sensitization of cytochrome oxidation. The latter reaction, signaled by an absorption increase at 403 mμ and a decrease at 420 mμ, was studied in a suspension of intact cells. The cells were suspended in 30% aqueous albumin to eliminate turbidity. Results are shown in Figs. 8a (bleaching of P870) and 8b (oxidation of cytochrome). A comparison of absorption and excitation spectra in Fig. 8a shows that quanta absorbed by BPh were 50% as effective, in promoting the bleaching of P870, as quanta absorbed by BChl. The spectra of Fig. 8b do not afford an accurate estimate of relative quantum efficiencies, but they do show that BPh, as well as BChl, was active in sensitizing the light-induced oxidation of cytochrome.

The results of Figs. 7 and 8 indicate that energy was transferred from BPh to P870 (promoting bleaching of P870) more efficiently than from BPh to BChl (promoting BChl fluorescence). This might reflect merely a variation from one preparation to another. It does suggest, however, that there are direct pathways of energy transfer from BPh to P870 as well as pathways going through B870.

DISCUSSION

Green plants and algae possess a pigment, P700, that appears to act as an agent for trapping excitation energy and using this energy to transfer electrons from cytochrome to an electron-acceptor (possibly plastoquinone)[3-7]. Recent evidence[8-12] indicates that an analogous system exists in purple bacteria, with BChl in place of chlorophyll *a*, P870 in place of P700, and perhaps ubiquinone in place of plastoquinone.

The experiments described here have established P870 as a distinct component of *R. spheroides* chromatophores. P870 can be isolated from the major ("light-harvesting") BChl component, at least spectrophotometrically. P870 appears to be BChl in a specialized environment that renders it non-fluorescent. In organic solutions it has absorption bands at about 590 and 770 mμ.

The demonstrated possibility of converting nearly all of the BChl in chromatophores to BPh, while leaving the reversible light-reactions of P870 and cytochrome intact, raises a question as to the essential role of chlorophyll in photosynthesis. If it proves possible to demonstrate complete photosynthesis in pheophytinized *R. spheroides* cells, it will follow that the specific requirement for BChl is restricted to its function in the role of P870.

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REFERENCES

[1] J. FRANCK, *Proc. Natl. Acad. Sci. U.S.*, 44 (1958) 941.
[2] M. CALVIN, *J. Theoret. Biol.*, 1 (1961) 258.
[3] B. KOK, *Biochim. Biophys. Acta*, 48 (1961) 527.
[4] H. T. WITT, A. MÜLLER AND B. RUMBERG, *Nature*, 192 (1961) 967.
[5] W. BUTLER, *Arch. Biochem. Biophys.*, 93 (1961) 413.
[6] L. N. M. DUYSENS, J. AMESZ AND B. M. KAMP, *Nature*, 190 (1961) 510.
[7] M. KLINGENBERG, A. MÜLLER, P. SCHMIDT-MENDE AND H. T. WITT, *Nature*, 194 (1962) 379.
[8] W. ARNOLD AND R. K. CLAYTON, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 769.
[9] B. CHANCE AND M. NISHIMURA, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 19.
[10] W. J. VREDENBERG AND L. N. M. DUYSENS, *Nature*, 197 (1963) 355.
[11] R. K. CLAYTON, *Biochem. Biophys. Res. Commun.*, 9 (1962) 49.
[12] R. K. CLAYTON, *Photochem. Photobiol.*, 1 (1962) 201.
[13] R. K. CLAYTON, *Photochem. Photobiol.*, 1 (1962) 305.
[14] J. C. GOEDHEER, *Biochim. Biophys. Acta*, 38 (1960) 389.
[15] L. N. M. DUYSENS, W. J. HUISKAMP, J. J. VOS AND J. M. VAN DER HART, *Biochim. Biophys. Acta*, 19 (1956) 188.
[16] R. K. CLAYTON AND C. SMITH, *Biochem. Biophys. Res. Commun.*, 3 (1960) 143.
[17] R. K. CLAYTON, *Biochim. Biophys. Acta*, 37 (1960) 503.
[18] J. W. WEIGL, *J. Am. Chem. Soc.*, 75 (1953) 999.
[19] C. S. FRENCH, *J. Gen. Physiol.*, 23 (1940) 483.
[20] A. S. HOLT AND E. E. JACOBS, *Am. J. Botany*, 41 (1954) 718.
[21] G. COHEN-BAZIRE, W. R. SISTROM AND R. Y. STANIER, *J. Cellular Comp. Physiol.*, 49 (1957) 25.