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REACTION CENTER PREPARATIONS OF *RHODOPSEUDOMONAS SPHEROIDES*:

ENERGY TRANSFER AND STRUCTURE

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SUMMARY

1. A procedure for the preparation of a reaction center fraction from wild type *Rhodopseudomonas spheroides* is described. The process involves two subsequent detergent treatments. The particles were purified down to a protein weight of 120000 daltons. They contain little cytochrome and 1.2 moles of ubiquinone per mole of P870. The negative absorption change in the light *minus* dark difference spectrum is not inconsistent with the assumption that 1 mole of ubiquinone is reduced per mole of photooxidized P870.

2. Bacteriopheophytin (with an absorption maximum at 756 nm) transfers energy to the reaction center bacteriochlorophyll (Bchl) with a high quantum yield. Fluorescence emission spectra indicate that this transfer occurs *via* P800.

3. If P870 is reduced, P800 transfers energy to P870 with high quantum yield. The fluorescence emission of P800 was not detectable even when P870 was oxidized, indicating that the fluorescence yield of P800 was less than $4 \cdot 10^{-5}$. An attempt was made to calculate the distance between P800 and P870, using the Förster equations of energy transfer by dipole–dipole interaction. The results are not inconsistent with earlier conclusions that P800 and P870 form a trimer.

4. In addition to normal reaction center particles we prepared particles which contained a small amount of bulk Bchl. One of these particles contained B850 in addition to reaction center Bchl. The B850 was at least partly active in energy transfer to P870.

5. The present findings were combined into a working hypothesis concerning the substructure of a photosynthetic unit of *Rps. spheroides*.

INTRODUCTION

During the last few years much attention had been paid to the treatment of bacterial chromatophores with agents which yield so-called reaction center particles,

Abbreviations: Bchl, bacteriochlorophyll; B800, B850 and B870, light-harvesting Bchl with absorption maxima around 800, 850 and 870 nm, respectively; P800 and P870, reaction center Bchl with absorption maxima at 800 and 870 nm, respectively.

i.e. subchromatophore particles which contain P870 (the reaction center bacteriochlorophyll (Bchl) type which is capable of undergoing reversible light-induced oxidation), but which do not contain the bulk or light-harvesting Bchl components normally present in chromatophores. In these relatively simple systems, spectral investigations can be done more precisely, and one may hope to find the primary electron acceptor of P870 through successive purification steps of the particles.

KUNTZ *et al.*[1] treated chromatophores with iridic chloride, which bleaches most of the bulk Bchl of *Chromatium* D and *Rhodospirillum rubrum*, and all of the bulk Bchl in *Rhodopseudomonas spheroides*. BEUGELING[2] in an independent study, bleached the bulk Bchl of *R. rubrum* chromatophores completely with ferricyanide. In all these cases, the reaction center Bchl remained fully active.

Another line of investigation is treatment of chromatophores with detergents. BRIL[3] used Triton X-100 to disrupt chromatophores of *Rps. spheroides* into two types of fragments, one containing B800 and B850 and the other containing B870. GARCIA *et al.*[4–7] working with four other species of bacteria added Triton X-100 to chromatophores and obtained a particulate fraction, consisting of small particles, sometimes in a linear or planar arrangement, and a membrane fraction. The latter was always photochemically less active than the particulate fraction, and in some cases was not active at all. THORNBER[8] used the detergent sodium dodecyl sulphate to fragment chromatophores of Chromatium. After separation on a hydroxylapatite column he obtained particles of different pigment composition, achieving a partial separation of the various Bchl types. One of the particles contained P870.

REED AND CLAYTON[9] and REED[10] isolated reaction center particles from a carotenoidless mutant of *Rps. spheroides* by means of Triton X-100, followed by density-gradient centrifugation. At about the same time GINGRAS AND JOLCHINE[11] reported the isolation of a reaction center particle from a carotenoidless mutant of *Rsp. rubrum*, using Triton X-100. THORNBER *et al.*[12] made a reaction center preparation from a wild strain of *Rps. viridis*, using sodium dodecyl sulphate as detergent; and JOLCHINE *et al.*[13] prepared reaction center fractions from normal *Rps. spheroides*, using cetyl trimethyl ammonium bromide.

We wish to report on the isolation of a functionally active reaction center preparation from a wild strain of *Rps. spheroides*. The aim of this work was to isolate a photochemically active particle of the smallest possible size. At present the particles have been purified down to a protein weight of 120000 daltons per mole of P870. They contain little cytochrome (0.25 mole per mole of P870) and 1.2 moles of ubiquinone per mole of P870. 1 mole of ubiquinone appears to be reduced per mole of photooxidized P870. Measurements of fluorescence and light-induced absorbance changes were done to get some insight in the mechanism of energy transfer from P800 to P870.

In the course of this work various combinations of the different light-harvesting Bchl types and reaction center Bchl have been obtained and possible consequences concerning the organization of the photosynthetic unit will be discussed.

MATERIALS AND METHODS

Preparation of the reaction center particle

Rps. spheroides was cultured anaerobically in a medium after COHEN-BAZIRE

et al.[14] supplied with yeast extract and peptone. After 3–4 days of growth the cells were harvested by centrifugation, washed in 0.05 M Tris (pH 8.0) and stored at liquid nitrogen temperature until use.

Fragmentation of the cells was carried out by sonication with a Branson type S125 sonifier during 10 min at 8 A. The temperature was not allowed to rise above 8° during sonication. In some cases the cells were passed through a French press at 5000 kg/cm² after precooling of the cell. The suspension was centrifuged for 30 min at 20000 × *g* to remove cell debris. CsCl and $MgSO_4$ were added to the supernatant to give final concentrations of 27 % and 0.05 M, respectively, and the suspension was centrifuged for 1.5 h at 144000 × *g*. The top layer was diluted, sedimented at 200000 × *g*, resuspended in 0.05 M Tris (pH 8.0) containing 0.01 M $MgCl_2$ and dialyzed overnight against the same buffer.

The absorbance of the chromatophore suspension at 590 nm was adjusted to 12.5/cm and 0.11 vol. of 3 % sodium dodecyl sulphate was added. After 2 h of incubation at room temperature NaCl was added to give a final concentration of 0.2 M and the suspension was layered on a 0.5 M sucrose solution in 0.05 M Tris (pH 8.0) supplied with 0.2 M NaCl. After 4 h of centrifugation at 200000 × *g* the chromatophores were sedimented and a greenish band containing the reaction center particles remained in the supernatant layer on top of the sucrose solution. This treatment was also successful with chromatophores of *Rps. viridis* (*cf.* re . 10), but not *Rsp. rubrum*. The reaction center particles were dialyzed for 2 days against three 25-vol. quantities of 0.05 M Tris (pH 8.0) to which 0.01 M $MgCl_2$ was added. The reaction center fraction was purified further by centrifugation at 200000 × *g* on a 0.4–2.0 M linear sucrose density gradient. This procedure resulted in the separation of a small fraction of less purified reaction center. The purified reaction center was dialyzed against 0.05 M Tris (pH 8.0) containing 0.01 M $MgCl_2$, to remove sucrose. The reaction center preparation purified in this way will be called hereafter SDS-RC.

A further purification was obtained by a procedure analogous to the so-called AUT-treatment used by LOACH *et al.*[15] for chromatophores. SDS-RC preparations were adjusted to an absorbance of 0.5–1 per cm at 803 nm and layered on top of a linear 0.1–1.0 M sucrose gradient in 0.05 M Tris containing 0.3 % Triton X-100 and 1 M urea. The pH had been adjusted to 10.0 after the addition of urea. After 6 h of centrifugation at 200000 × *g* the original preparation had been separated into an upper bright green band and a lower brown band. Both bands were situated about halfway down the sucrose gradient. The lower band consisted of the purified reaction center preparation and contained about 85 % of the total amount of Bchl. The total recovery of Bchl in the two fractions was about 80 % of the original amount. Both fractions were dialyzed against three 25-vol. quantities of 0.05 M Tris (pH 8.0) containing 0.01 M $MgCl_2$. The reaction center particle purified in this way will be denoted AUT-RC.

Chemical methods

Protein was measured by the method of LOWRY *et al.*[16]. Bchl was determined by extraction with cold acetone–methanol (7:2, by vol.) using an extinction coefficient of 75 $mM^{-1} \cdot cm^{-1}$ (ref. 17). In reaction center fractions Bchl was also determined *in vivo*, using the extinction values given by CLAYTON[18] and by BOLTON *et al.*[19]. Cytochrome was measured with the ferrihemochrome method as used by CUSANOWITCH

AND KAMEN[20]. Carotenoid (mainly spheroidene) was estimated using an extinction coefficient of 150 $mM^{-1} \cdot cm^{-1}$ at 473 nm (*cf.* ref. 21). Iron was determined with the ferrous *o*-phenanthroline complex method[22] after a mineralization procedure as used by KONINGS[23]. The sample was concentrated to near dryness by heating at 120°, then 0.2 ml conc. H_2SO_4 (Merck, p.a.) was added and the samples were heated at 170° for 2 h. They were then heated on a Bunsen burner while $HClO_4$ was added dropwise until a bright yellow colour appeared and disappeared again. Copper was determined as follows: after the same wet-ashing procedures as used for iron, ^{63}Cu was transformed into ^{64}Cu by neutron irradiation. The characteristic radiation of 0.51 MeV which ^{64}Cu emits as it decays was measured with an NaI detector coupled with a γ-spectrometer. Ubiquinone was determined by the method of PUMPHREY AND REDFEARN[24] as modified by TAKAMIYA AND TAKAMIYA[25].

For polyacrylamide disc gel electrophoresis the buffer system given by SMITH[26] was used. The acrylamide concentration was 3.75 %. The samples were layered on the gel in tubes of 7.5 cm length and 0.5 cm diameter and subjected to electrophoresis at 250 V (5 mA per tube). Urea or detergent was present both in the sample and in the gel as indicated.

Physical methods

Absorbance spectra were measured on a Cary model 14 R spectrophotometer.

Light-induced absorbance changes were measured with a split-beam differential spectrophotometer described earlier[27].

Fluorescence emission spectra were measured in 1-mm cuvettes with an apparatus similar to the one described earlier[28]. The excitation light from a 600-W tungsten–iodine lamp was chopped at 50 Hz and passed through a Schott 596 AL filter (band width 20 nm). The absorbance of the sample at 803 nm was 0.14/mm or less. The emitted light was analyzed by a Bausch and Lomb monochromator. The slits were adjusted to give a band width of 8 nm. An S1 photomultiplier was attached to the exit slit. Stray excitation light was cut off by a Schott RG 10 and a Kodak Wratten 89B filter. The spectra were corrected for the wavelength-dependent sensitivity of the detecting system and for reabsorption of the emitted light. The intensity of the excitation light was 60 $nE \cdot cm^{-2} \cdot sec^{-1}$.

RESULTS

Chemical data

The preparations which will be discussed were all photochemically active: P870 could be bleached completely and reversibly and with a high quantum yield.

Sodium dodecyl sulphate treatment of the chromatophores, followed by ultracentrifugation, resulted in the extraction of about 75 % of the total amount of P870. The extracted chromatophores contained the remaining 25 %. The amount of P870 was measured by the maximum light-induced absorption decrease at 600 nm, as will be discussed later.

Table I shows the results of chemical analysis on SDS-RC and AUT-RC particles compared with chromatophores and with the results of JOLCHINE *et al.*[13] and REED[10]. The chemical composition of the reaction center particle obtained by Reed is such that it resembles that of chromatophores except that light-harvesting Bchl is not

present[29]. This was not the case with our preparations: the cytochrome and ubiquinone content relative to the amount of P870 was lower in reaction center preparations than in chromatophores. The oxidized *minus* reduced difference spectra of the cytochrome present in the SDS-RC and AUT-RC preparations showed maxima at 416, 520 and 550 nm as measured with the ferrihemochrome method; and at 420, 520 and 550 nm when measured *in vivo*. More than 50 % of the total amount of ubiquinone was retained in the extracted chromatophores after the sodium dodecyl sulphate treatment, and the amount of ubiquinone found in SDS-RC preparations was comparable to that found by JOLCHINE *et al.*[13]. The amount of protein in SDS-RC preparations was the same as the amount found by JOLCHINE *et al.*[13], which may be a reflection of the fact that in both cases an ionic detergent has been used; REED and coworkers[9,10,29], on the other hand, used Triton to isolate the reaction center particles (see DISCUSSION).

TABLE I

CHEMICAL COMPOSITION OF CHROMATOPHORES AND REACTION CENTER PARTICLES PREPARED FROM *Rps. spheroides*

Quantities are expressed in moles per mole of P870. The first three columns give the composition of the reaction center particles obtained by REED[10], using 2.3 % and 1 % Triton, respectively, and by JOLCHINE *et al.*[13]. Cytochrome *b* was determined *in vivo* by the difference spectrum (dithionite-reduced *minus* no additions). For other methods see section MATERIALS AND METHODS.

	Ref. 10, Triton		*Ref. 13*			
	2.3 %	*1 %*	*Normal RC*	*Chromatophores**	*SDS-RC**	*AUT-RC**
P870	1	1	1	1	1	1
Iron	5.4	16		N.D.	20**	N.D.
Cytochrome *b*562	0.8	1.8	0	0.8**	<0.02	<0.02
Cytochrome *c*	0.0	1	0.47	0.95	0.90	0.25
Ubiquinone	7.4	13	5.5	8–12	3.3	1.2
Copper	6.2	9		N.D.	0.5**	N.D.
Protein***	$4.1 \cdot 10^5$		$2.05 \cdot 10^5$	$6 \cdot 10^5$	$2.0 \cdot 10^5$	$1.2 \cdot 10^5$
Bulk Bchl				30	<0.001§	<0.001§

* Average from 3 determinations. Preparations purified by sucrose density gradient centrifugation (see MATERIALS AND METHODS).
** One determination.
*** Expressed in g per mole of P870.
§ Determined by fluorescence measurements.
N.D., not determined.

Table I shows that AUT-RC particles had a relatively low protein content, little cytochrome and, notably, 1.2 moles of ubiquinone per mole of P870. On the other hand, the upper fraction obtained by the AUT treatment (see MATERIALS AND METHODS) had a relatively high protein content (250000–300000 g per mole of P870), 1.6 moles of cytochrome *c* and 8–13 moles of ubiquinone per mole of P870. The recovery of protein and ubiquinone in these two fractions was about 80 % of the initial amounts; that of cytochrome *c* was about 40 %.

The AUT-RC particles ran as a single band during polyacrylamide disc gel electrophoresis in the presence of 0.1 % sodium dodecyl sulphate. It was separated into three bands in the presence of 0.3 % sodium dodecyl sulphate *plus* 8 M urea

(*cf.* ref. 30). When 0.2 % digitonin was present the AUT preparation splitted into two coloured bands. Other detergents appeared to be unsuccessful in this respect. Work is in progress to identify the different bands thus obtained.

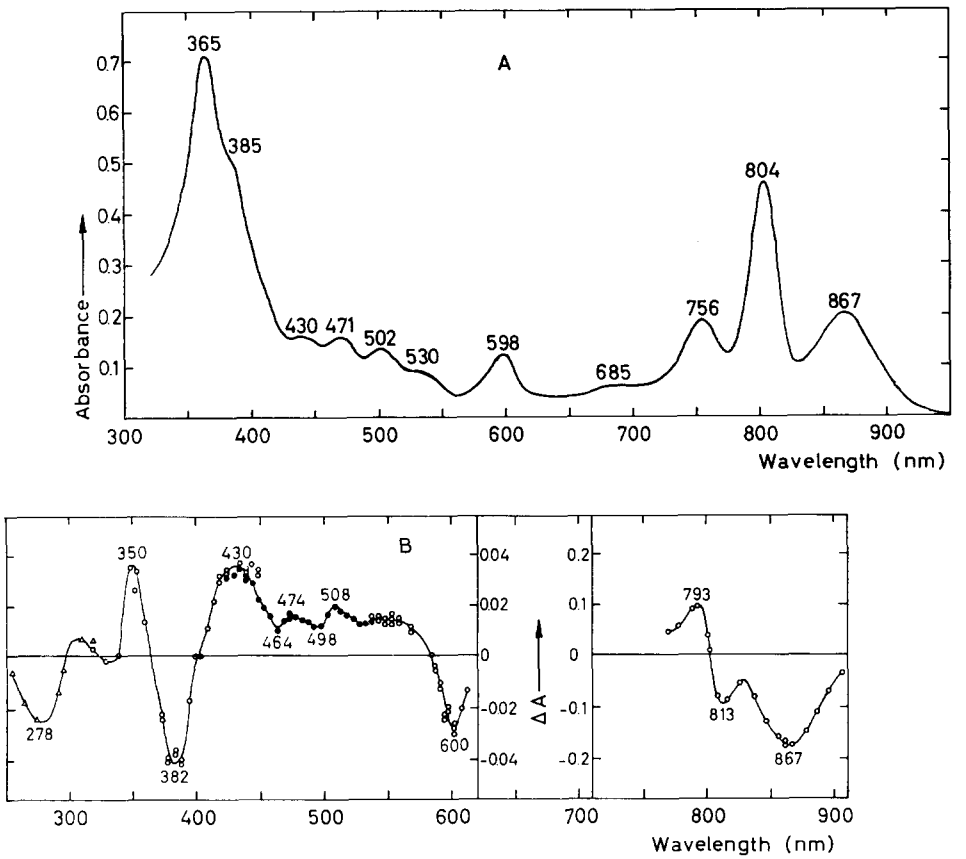

Fig. 1. (A) Absorption spectrum of an AUT-RC preparation. (B) Light *minus* dark absorption difference spectrum (steady state) of a normal reaction center preparation. AUT-RC particles showed the same difference spectrum. The scales of (A) and (B) have been normalized so that (B) represents the maximum light-induced difference spectrum of the sample shown in (A). Light regime: 5 sec light, 90 sec dark. Actinic light: 596 nm (band width 20 nm), intensity 0.7 nE·cm^{-2}·sec^{-1} for the ultraviolet and infrared measurements; infrared light (cut off by Schott RG 20.2 and Corning CS 7-69 filters), intensity approx. 0.2 nE·cm^{-2}·sec^{-1} for the visible measurements. A time-averaging technique was used for the ultraviolet measurements. No additions. The different symbols refer to 3 samples which were normalized for equal absorption change at 867 nm.

Absorption and absorption difference spectra

The SDS-RC preparations had absorption spectra similar to those published before[10,13]. Fig. 1A shows the absorption spectrum of an AUT-RC preparation. The ultraviolet absorption is not shown, since the Triton present in these preparations absorbed strongly in this region. In SDS-RC preparations the ratio of the absorbances at 280 and at 870 nm was correlated to the protein/P870 ratio as determined by the Lowry method. At a protein/P870 ratio of 200000 per mole of Bchl we found an $A$276 nm/$A$870 nm ratio of 7.8. In one SDS-RC preparation we found 96000 daltons

protein per mole of P870 by the Lowry method and there the $A$276 nm/$A$870 nm ratio was 4.6. These data are in fair agreement with data published earlier on ultraviolet absorption and protein content of reaction center preparations[10,13].

The maxima at 804 and 867 nm are due to P800 and P870, respectively. The maxima at 365 and 598 nm are due to both P800 and P870. The shoulder at 385 nm and the maxima at 536 and 756 nm are probably due to bacteriopheophytin, and the maxima at 440, 471 and 502 nm are due to carotenoid. The main difference with SDS-RC preparations is that the shoulder at 405 nm and the maximum at 685 nm are much lower in the AUT-RC than in SDS-RC preparations, suggesting that these bands are due to a degradation product of Bchl.

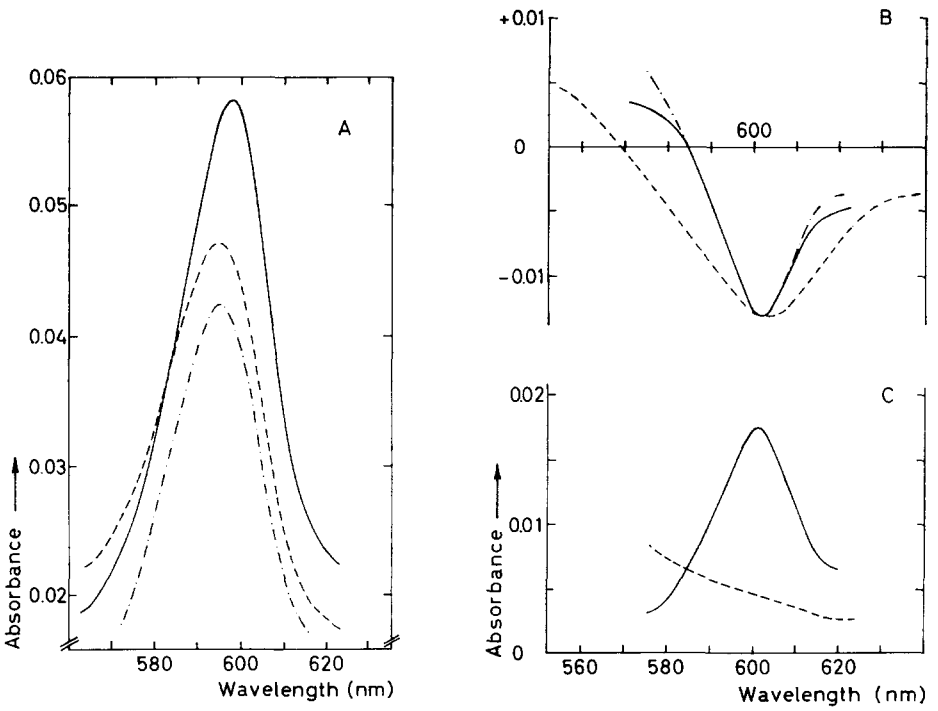

Fig. 2. (A) ———, absorption spectrum of an AUT-RC preparation, with P870 reduced (no additions); — — —, same with P870 oxidized (0.1 mM ferricyanide); –·—·–, absorption band due to P800. (B) (a) — — —, absorption difference spectrum (ferricyanide *minus* no additions) of Bchl in methanol (data from ref. 32); (b) –·—·–, same as (a) but the band width was narrowed by a factor 2.0 and the whole spectrum was shifted 2 nm towards shorter wavelengths; (c) ———, absorption difference spectrum (ferricyanide *minus* no additions) of the AUT-RC preparation shown in (A). (C) Absorption spectrum of P870 of the AUT-RC shown in (A). ———, reduced; — — —, oxidized.

The light-induced absorption difference spectrum of an SDS-RC center preparation is shown in Fig. 1B. AUT-RC preparations showed the same difference spectrum. It is virtually the same as that obtained by REED[10] except that carotenoid red shifts are also present in our preparations. The occurrence of these shifts is apparently not dependent on whether the chromatophore membrane is intact. Assuming that the lipophilic substances of the reaction center particles are buried inside (*cf.* ref. 31),

these carotenoid band shifts are suggested to be reflection of, *e.g.* a change in pH or in polarity inside the reaction center particle upon oxidation of P870.

Fig. 2A shows the absorption band around 600 nm of an AUT-RC preparation with P870 in the reduced and in the oxidized state, respectively. In the latter case part of the absorption is due to oxidized P870. For the measurement of the fluorescence yields of P800 and P870, to be described in the next section, it was necessary to analyze the absorption band of the AUT-RC preparation around 600 nm into the absorption bands of P800 and P870. However, the absorption band of P870 could not be obtained directly. For this reason, the oxidized *minus* reduced absorption difference spectrum around 600 nm of an AUT-RC preparation was compared with the oxidized *minus* reduced difference spectrum of Bchl in methanol (data from ref. 32). See Fig. 2B. The difference spectrum obtained with Bchl in methanol can be made to correspond fairly well to the difference spectrum obtained with AUT-RC if (1) the band width of the former is narrowed by a factor 2, and (2) the position of the peak is shifted slightly. Since the absorption change of the AUT-RC preparation in this region was due to P870 oxidation[33], it was assumed that the absorption spectra of P870 (reduced and oxidized) can be obtained from the absorption spectra of Bchl in methanol[32] by carrying out the same transformations along the wavelength axis (see Fig. 2C). It was found that P870, when completely reduced, had a peak at 601 nm. The AUT-RC had a peak at 598 nm when P870 was completely reduced. The ratio of the peak heights of reduced P870 and reduced AUT was 0.3:1. The absorption peak of P800 was obtained by subtracting the absorption of P870 from the total absorption of the AUT-RC preparation. In this way an absorption band of P800 was found with a maximum at 595 nm (see Fig. 2A). If it was assumed that the peak height of P800 was twice that of reduced P870, then it appeared that the half width of the absorption band of P800 was 1.16 times the half width of reduced P870. This indicated that the method followed was not quite correct, but it certainly put an upper limit to the fractional absorption of P800. The remaining absorption which was not due to P800 and P870 was probably due to bacteriopheophytin. In an oxidized AUT-RC preparation this was estimated to be 7–10 % of the total absorption at 595 nm.

There is no unanimity concerning the nature of the absorption decrease around 275 nm. This change has often been ascribed to ubiquinone[34,2]. KE AND CHANEY[35], using *Chromatium* subchromatophore particles, were able to distinguish kinetically the dark decay of the light-induced 280-nm absorption changes and the 880-nm absorption change, respectively, suggesting that the former was due to ubiquinone. However, LOACH *et al.*[33] proposed that at least part of the ultraviolet absorption decrease is due to oxidation of P870. They showed that Bchl shows a decrease of absorption in this region upon oxidation *in vitro*. If it is assumed that the absorption change is only due to ubiquinone in our preparations, then a 1:1 relationship exists between the amount of ubiquinone reduced and the amount of P870 oxidized, assuming that the differential extinction coefficient of ubiquinone (oxidized *minus* reduced) at 275 nm is 12.2 $mM^{-1} \cdot cm^{-1}$ (ref. 36) and that the differential extinction coefficient of P870 is 93 $mM^{-1} \cdot cm^{-1}$ (ref. 19). The same relationship has been found in other reaction center preparations[2,10]. In this connection it is interesting to note that 1.2 moles of ubiquinone per mole of P870 were found in AUT-RC preparations (see previous section). Approximately the same amount was found by BEUGELING[2]. On the other hand, if the changes at 275 nm are due to both P870 and

ubiquinone, then of course the ratio of ubiquinone to P870 will be lower than 1:1. The results of TAKAMIYA AND TAKAMIYA[37] may support this possibility.

Transfer of light excitation energy in reaction center preparations

An investigation into the transfer of light energy from P800 and from bacteriopheophytin to P870 was carried out in three steps. In the first step we compared the quantum efficiencies of light absorbed by P800, bacteriopheophytin and P870, respectively, for the photooxidation of P870, in order to calculate the efficiency of the energy transfer from P800 and from bacteriopheophytin to P870. In the second step we measured the fluorescence yield of P800 and of P870. The yield of P800 appeared to be less than $4 \cdot 10^{-5}$. In the third step, using the above-mentioned data, we calculated the distance between P800 and P870, assuming that P800 transfers excitation energy to P870 *via* a Förster mechanism of resonance transfer.

Step 1. Quantum efficiencies for the photooxidation of P870

The maximum light-induced absorption decrease at 600 nm, due to P870 oxidation, was compared with the maximum light-induced decrease at 865 nm. Assuming that the differential extinction coefficient of P870 (reduced *minus* oxidized) at 865 nm is 93 $mM^{-1} \cdot cm^{-1}$ (ref. 19), a differential extinction coefficient of 15.3 $mM^{-1} \cdot cm^{-1}$ was derived for P870 at 600 nm. The initial quantum efficiency for P870 oxidation (measured at 600 nm) was independent of the actinic light intensity within the measured range (0.35–16 $nE \cdot cm^{-2} \cdot sec^{-1}$).

The quantum efficiency for P870 oxidation varied somewhat in normal reaction center preparations, but our results were in agreement with the data of BOLTON *et al.*[19].

Light of 872 nm had a quantum efficiency ranging from 0.91 to 1.26. Light of 803 nm seemed to be slightly less active than light of 872 nm. In order to eliminate systematical errors involved in these calculations (errors in the estimation of the molar extinction coefficient of P870, in the measurement of the actinic light intensity, and in the calibration of the magnitude of the absorption decrease) the quantum yield of 803 nm light was expressed relative to the quantum yield of 872 nm light for the bleaching at 600 nm. Putting the quantum yield of 872 nm light equal to 1, we derived a quantum yield of 0.89 ± 0.05 for 803 nm light as an average for 7 different preparations. This is taken to be the efficiency of the energy transfer from P800 to P870.

Bacteriopheophytin was present in all reaction center preparations studied so far. The amount was rather variable, but in the purest preparations the area under the absorption bands around 756 and 867 nm, respectively (plotted against wavenumbers) was approx. 0.9:1. The bacteriopheophytin that could not be removed appeared to transfer energy to reaction center Bchl with a high efficiency. Light of 764, 803 and 872 nm had a quantum efficiency of 1.21 ± 0.07, 0.87 ± 0.06, and 1, respectively, for the bleaching at 600 nm (average for three different preparations).

Step 2. Fluorescence yields of P870 and P800

Fig. 3 shows the absorption and fluorescence spectra of an SDS-RC preparation (AUT-RC preparations gave similar results). The excitation light was sufficiently strong to keep P870 completely oxidized when no additions were present. In the presence of low potential reductants (dithionite or high concentrations of ascorbate), which block electron transfer from P870 by reducing the primary electron acceptor,

P870 remained completely reduced in the excitation light (*cf.* ref. 38). The emission difference spectrum (reduced *minus* oxidized) around 910 nm, which is ascribed to P870, resembles the one obtained earlier[33]. The peak at 785 nm in the emission spectrum is due to bacteriopheophytin. As is seen from the figure, the emission at 785 nm is higher when P870 is reduced than when it is oxidized. If bacteriopheophytin transfers its energy directly to P870, one would expect the reverse. An increase in fluorescence of bacteriophytin upon reduction of P870 may be explained at least partly by assuming that bacteriopheophytin transfers its energy to P800. The shift towards longer wavelengths of the absorption band of P800 upon reduction of P870 causes a decrease in the overlap between the bacteriopheophytin emission band and the P800 absorption band. This will result in an increase of the ratio of the rates of de-excitation of bacteriopheophytin by emission and by energy transfer to P800, respectively.

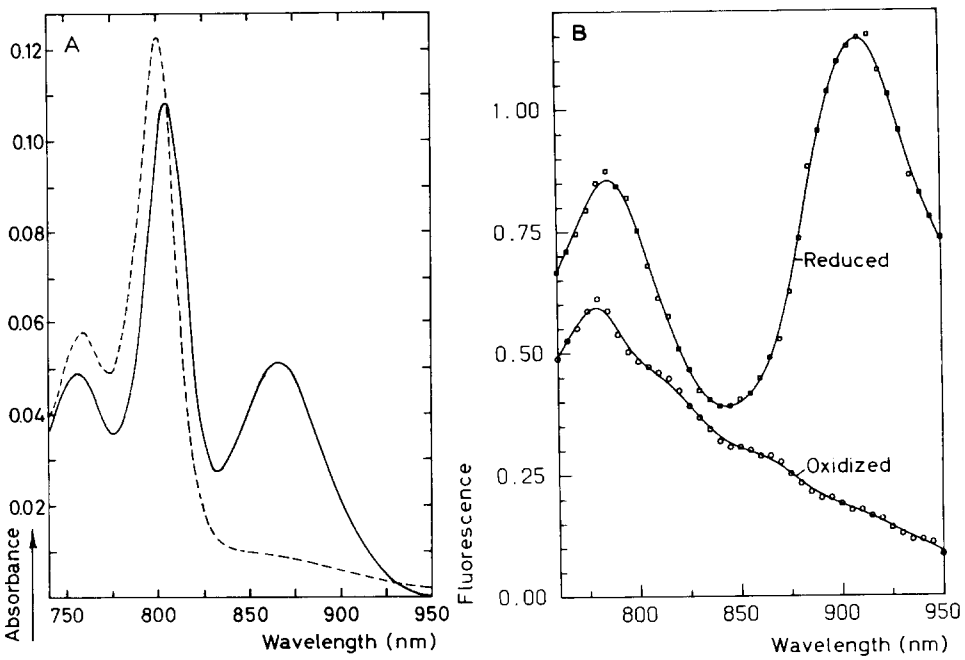

Fig. 3. (A) Absorption spectrum (A/mm) of an SDS-RC preparation. ———, no additions; — — —, 0.1 mM ferricyanide. (B) Fluorescence emission spectrum of the preparation shown in (A). Oxidized, no additions; reduced, 2 mM dithionite. Excitation light: 596 nm (band width 20 nm), intensity 60 nE·cm^{-2}·sec^{-1}. The measurements were done in 1-mm cuvettes. The absorbance of the samples was 0.12 per mm at 804 nm. A correction for stray excitation light was applied by subtracting the signals obtained with water in the cuvette. The remaining signal was corrected for reabsorption of the emitted light and for the wavelength dependent sensitivity of the detecting system. The curves were obtained with a smooth spline method using a standard deviation of 0.01.

We calculated the distance R between P800 and P870 on the assumptions (1) that the energy is transferred from P800 to P870 *via* dipole–dipole interaction, (2) that the rate of the energy transfer varies as R^{-6}, and (3) that a thermal equilibrium of the collisional vibration levels of the excited state on P800 is established before energy transfer to P870 takes place. With these assumptions the Förster equations can be applied.

We determined the fluorescence yield of P870 in reaction center preparations relative to the yield of light-harvesting Bchl in chromatophores. Assuming the latter value to be 3.0 % (*cf.* ref. 39), we arrived at a fluorescence yield of 0.15 % and 0.03 % for P870 in the presence and in the absence of low-potential reductants, respectively. This was in accordance with earlier obtained results[38].

An emission band of P800 was not detected in our spectra, not even when P870 was oxidized. Considering the sensitivity of the measuring system, this meant that the magnitude of the peak of the P800 emission under our measuring conditions was less than 0.04 times the magnitude of the peak of P870 emission which was observed in the presence of dithionite.

The assumption concerning thermal equilibrium of the excited state on P800 allowed us to calculate the expected emission spectrum of P800, using the Stepanow equation (ref. 40, as cited in ref. 41). This equation is valid for P870 (ref. 38 and own observations) and for B870 (unpublished results). The half width of the expected emission band of P800 was about 2 times smaller than the half width of the P870 emission band. This means that the total emission from P800 was less than 0.02 times the total emission from P870 in the presence of dithionite. Now, if P870 is reduced, light absorbed by both P800 and P870 and by bacteriopheophytin contributes to the P870 fluorescence (Fig. 2A, line ————). It was assumed that if P870 is oxidized, only light absorbed by P800 and bacteriopheophytin contributes to P800 fluorescence (Fig. 2A, line —·—). Taking this into account, we concluded that the fluorescence yield of P800 was less than $4 \cdot 10^{-5}$ even when P870 was oxidized.

Step 3. Calculations

The distance R_0 between two molecules which transfer energy to each other *via* the Förster mechanism is defined as the distance at which the rate of de-excitation by energy transfer (Kt) equals the rate of deexcitation by fluorescence (Ke). Then

$$\frac{K_t}{K_e} = \left(\frac{R_0}{R}\right)^6$$

in which R is the actual distance between the molecules.

The quantity Kt/Ke was calculated from

$$\frac{K_t}{K_e} = \frac{Q}{(1-Q)\varphi} \quad (cf.\ \text{ref. } 42)$$

in which Q is the quantum yield for the energy transfer and φ is the fluorescence yield in the absence of energy transfer. With $\varphi < 4 \cdot 10^{-5}$ and $Q = 0.89$ (average) we found that $R < 0.13\ R_0$. (It is assumed that the average rate constant of the internal conversion processes of the excited state on P800 is independent of the redox state of P870. We will return to this later.)

The distance R_0 was calculated from the overlap between the emission band of P800 and the absorption band of P870, using the Förster equation (Eqn. 9.1 in ref. 42). This equation contains an angular factor k^2 which depends on the position of the red dipoles of P800 and P870. Fluorescence polarization measurements (ref. 43 and own observations) indicate an angle of approx. 25 % between the red dipoles of P800 and

P870. This means that the angular factor may vary from 0.81 to 3.24 and so R_0 varies from 77 to 94 Å and $R < 10$ Å if the red dipoles of P800 and P870 are nearly parallel; $R < 12$ Å if the red dipoles of P800 and P870 are nearly in line.

The intrinsic life time of the fluorescence from P800 was calculated from the absorption spectrum and the expected fluorescence spectrum of P800, according to Eqn. 1 of ref. 38. Contrary to ZANKEL *et al.*[38], we arrived at an intrinsic life time of 31 nsec for both P870 and P800. This meant that the life time of the P800 fluorescence was less than $1.2 \cdot 10^{-12}$ sec, whether P870 was reduced or oxidized. This is an extremely short life time.

The low fluorescence yield of P800 may be ascribed to energy transfer to P870 if the latter is reduced. Why then is the fluorescence yield of P800 so low when P870 is oxidized? Dimer formation can be the cause of lowering of the fluorescence yield, but circular dichroism measurements[44] indicated no interaction between the two molecules of P800 when P870 is oxidized. One might suppose that oxidized P870, too, quenches the fluorescence of P800. In that case the average rate constant of internal conversion processes of the excited state on P800 is not independent of the redox state of P870, and

$$\frac{K_t}{K_e} = \left(\frac{R_0}{R}\right)^6 = \frac{Q}{\varphi'}$$

in which φ' is the fluorescence yield of P800 in the presence of energy transfer. Then $R < 0.18\ R_0$, but on the other hand, in that case the oxidized P870 has to be at a very short distance of P800 anyway to quench the fluorescence from P800 within 10^{-12} sec.

A further correction may be necessary because the simple dipole approximation is not correct at distances that are comparable to the dimensions of the system of conjugated bonds of the individual molecules. In summary, the assumption of "slow" energy transfer (see above) leads to the result that P800 transfers its energy to P870 within $1.2 \cdot 10^{-12}$ sec along a distance which may be less than 10–12 Å. This result may be inconsistent with the assumption of "slow" energy transfer. They are not inconsistent with the result obtained by SAUER *et al.*[44], that P800 and P870 form a trimer.

In addition to the reaction center preparations discussed so far, we obtained, by a modification of the procedure outlined in MATERIALS AND METHODS, reaction center preparations which contained some residual B850 or B870 in addition to the reaction center Bchl. These preparations were investigated in the same way as indicated above, namely (1) by comparing the quantum efficiencies of light absorbed by B850, or B870, with that of light absorbed by P800 and P870, for the photooxidation of P870, and (2) measuring the fluorescence yield of B850, or B870, when P870 was reduced and when it was oxidized. The results indicated that the residual B850 in these preparations transferred energy to P870 (with an efficiency of roughly 50 %), but that the residual B870 did not. More details will be given in ref. 45.

DISCUSSION

We attempted to correlate the results discussed so far in a working scheme for the substructure of the photosynthetic unit of *Rps. spheroides*. This scheme was

designed primarily to fit the following observations. (1) Our chromatophores have a protein weight of $60 \cdot 10^4$ daltons per mole of P870; the particles obtained by LOACH *et al.*[15] from *Rps. spheroides* chromatophores have a protein weight of $10 \cdot 10^4$–$15 \cdot 10^4$. The latter particles contained probably each at least one complete photosynthetic unit, since no Bchl was lost during the preparation and all Bchl species transferred energy to P870 with a high efficiency[15]. (2) REED *et al.*[29] obtained reaction center particles from *Rps. spheroides* with a protein weight of $44 \cdot 10^4$ per mole of P870. (3) The SDS-RC particles obtained by us have a protein weight of $20 \cdot 10^4$ daltons per mole of P870 (*cf.* ref. 13). This can be reduced to $12 \cdot 10^4$ daltons per mole of P870 by AUT treatment; and FEHER *et al.*[30] obtained a reaction center particle from *Rps. spheroides* of about $4 \cdot 10^4$. This particle could in turn be split into two particles each of about $2 \cdot 10^4$ (ref. 30). These data may be combined into a scheme such as the one given in Fig. 4, which visualizes the protein partition of a photosynthetic unit of *Rps. spheroides* and the lines along which it can be splitted into smaller fragments by the indicated detergent treatments.

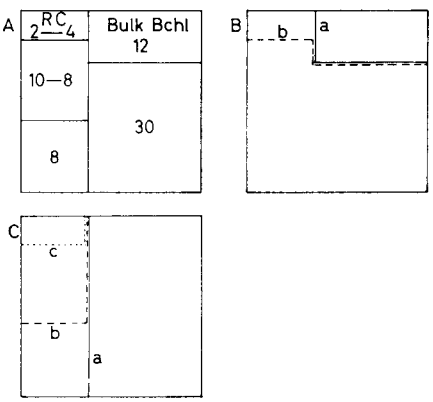

Fig. 4. (A) Scheme of the protein partition in a photosynthetic unit of *Rps. spheroides*. The numbers indicate the protein weight (daltons $\times 10^{-4}$) of the different subunits. (B) Hypothesized separation lines, corresponding to the lines given in (A), obtained (a) solid line, when using Triton to disrupt chromatophores of a carotenoidless mutant[9,10,29]; (b) dashed line, when using Triton and urea at alkaline pH to disrupt chromatophores of normal bacteria[15]. (C) Hypothesized separation lines obtained (a) solid line, when treating chromatophores of normal bacteria with cetyl trimethyl ammonium bromide[13] or with sodium dodecyl sulphate (this report); (b) dashed line, when treating SDS-RC preparations with Triton and urea at alkaline pH (this report); (c) dotted line, after gel electrophoresis in sodium dodecyl sulphate of a reaction center preparation[30].

According to this figure the bulk Bchl (about 30 moles per mole of P870 under our culturing conditions) is attached to $15 \cdot 10^4$ daltons of protein, in agreement with the data from LOACH *et al.*[15]. There are a few observations which are not in agreement with this notion. First, after sodium dodecyl sulphate treatment of chromatophores to remove reaction center particles, the residual material has a protein/Bchl ratio of 9000–10000 daltons per mole of Bchl (unpublished results), and so far we have not been able to diminish that ratio. Second, the photochemically active particles obtained by THORNBER[8] from *Chromatium* chromatophores had a similar protein/Bchl ratio. In this respect the results obtained by THORNBER AND OLSON[46] may be relevant. They obtained Bchl–protein complexes from green bacteria, which had a protein weight of about $16 \cdot 10^4$ daltons per particle and which consisted of 4 subunits, each

with a protein weight of about $3.7 \cdot 10^4$ daltons. One subunit contained 5 molecules of Bchl. However, it may be significant that the reaction center subunit obtained by FEHER *et al.*[30] had a particle weight of about $4 \cdot 10^4$ daltons and that it could be split into two halves of about $2 \cdot 10^4$ daltons each. We consider that it is not unlikely that a reaction center particle (containing 3 moles of Bchl and perhaps 1 mole of bacteriopheophytin) resembles a "bulk" Bchl-protein complex in its protein/Bchl ratio. It therefore seems attractive to hypothesize a substructure of the photosynthetic unit of *Rps. spheroides* consisting of 1 reaction center and about 6 bulk Bchl subunits. Each subunit would contain 5 bulk Bchl molecules and have a protein weight of $2 \cdot 10^4$ daltons, which would account for the protein content of the particles obtained by LOACH *et al.*[15].

Several other ways are known at present to split chromatophores of wild type *Rps. spheroides* into smaller fragments. With Triton two kinds of particles can be obtained, one containing B800 and B850, and the other containing B870 and reaction center[3]; it is possible to extract the reaction center from the latter particle by means of sodium dodecyl sulphate (unpublished results). Furthermore it has been shown in the present work that sodium dodecyl sulphate splits chromatophores into a fraction containing most of the bulk Bchl originally present (only B800 disappears largely as a result of sulphate sodium dodecyl sulphate treatment) and a fraction which contains reaction center particles and, under certain conditions, some residual B850 or B870.

These results suggest that the bulk Bchl is contained in subunits which contain either B870 or B800 and B850. (To our knowledge no spatial separation between B800 and B850 has been reported, only selective bleaching of B800.)

It is easy to visualize a configuration of subunits such that a reaction center unit can remain attached either to a B870 subunit, as was the case after Triton treatment[3] or after a modified sodium dodecyl sulphate treatment (this report) of chromatophores, or to a B800/B850 subunit, of which the B800 has been bleached, as was the case after a modified sodium dodecyl sulphate treatment of chromatophores (this report).

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