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PURIFICATION AND PROPERTIES OF A PHOTOSYNTHETIC REACTION CENTER ISOLATED FROM VARIOUS CHROMATOPHORE FRACTIONS OF *RHODOPSEUDOMONAS SPHEROIDES* Y*

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SUMMARY

1. Crude chromatophores, ribosome-free chromatophores and light particles, isolated from *Rhodopseudomonas spheroides* Y cells deficient or not deficient in iron, were used as source material for the preparation of reaction centers. Isolation was performed by incubation with cetyltrimethylammonium bromide and differential centrifugation; the purification was based upon either centrifugation or on $(NH_4)_2SO_4$ precipitation and gel filtration.

2. In all cases, the purified reaction center contained the photo-active bacteriochlorophylls P 865 and P 800 (in a 1:2 ratio) without any light-harvesting bacteriochlorophyll; carotenoids and bacteriopheophytin were present. The yield (P 865 recovered from 100 bacteriochlorophylls) and the P 865 content (expressed per g protein) varied with the source material; the highest values were obtained when starting from low-iron light particles, the lowest when starting from low-iron crude chromatophores.

3. The size of the particle bearing the reaction center was independent of the source material; the particle weight, determined by gel filtration as well as by equilibrium sedimentation, was about 150000. The midpoint potential of the P 865 species was 525 mV at pH 7.5.

4. Some components of the purified reaction center from crude chromatophores, and their ratios, were: in low iron conditions, P 865:ubiquinone:*c*-type heme:non-heme iron, 1:8.6:0.12:0.2; in normal conditions, P 865:ubiquinone:*c*-type heme:non-heme iron, 1:8:1.5:0.5.

INTRODUCTION

During the last 3 years, the separation by means of a detergent of the photochemical reaction center from the light-harvesting bacteriochlorophylls has been

Abbreviation: CTAB, cetyltrimethylammonium bromide.

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performed in a variety of organisms, *e.g.* carotenoid-less mutants of *Rhodopseudomonas spheroides* (R-26)[1] and *Rhodospirillum rubrum* (G9)[2], and carotenoid-containing strains of *Rhodopseudomonas spheroides* (Y)[3] and *Rhodopseudomonas viridis*[4]. Partial separation has been reported for *Chromatium*[5]. In all cases such reaction center fractions are particulate; apart from *Chromatium*, they are freed from the light-harvesting bacteriochlorophylls but still contain, besides the photoactive bacteriochlorophyll, bacteriopheophytin in particular, whose significance is unknown. They also contain what appear to be residues of the electron transport chain: ubiquinone, cytochromes, iron and copper have been detected and quantified in several cases. Depending on the organism, they may or may not be kept in solution without a detergent; their particle weight has been reported to be either variable, on account of aggregation phenomena (*Rps. spheroides* Y, *Rps. viridis*), or in the order of 650000 (*Rps. spheroides* R-26, *Chromatium*). How their resolution depends on source material, nature of detergent, the purification procedures, *etc.* is still an open question.

We recently described the isolation of a crude reaction center fraction from *Rps. spheroides* Y light particles, obtained from cells deficient or not deficient in iron[3]. Continuing this work, we have attempted to further purify the preparations in order to obtain more precise information as to particle weight and composition. In addition, various chromatophore fractions were used as starting material, *i.e.* crude chromatophores, ribosome-free chromatophores, and "light" particles. The results indicate that such variations, although affecting the specific activities of the purified reaction centers, do not modify their spectral characteristics, nor the particle weight which is about 150000. Such a small size of the reaction center particles, as well as the low amount of some electron transfer compounds bound to them, indicate that in this organism the reaction center particle can be isolated as a complex consisting of a few components.

MATERIAL AND METHODS

Cultures and particle preparation

Rhodopseudomonas spheroides strain Y was grown in 10-l bottles at 33° as described elsewhere[6]. The synthetic "L medium" was used to grow "low-iron" cells, whereas the "L, 17 μM iron medium" provided bacteria grown in the presence of excess iron. Stationary-phase cells grown for 36–48 h at 8000 lux were routinely used in 50-l culture batches.

The fractionation of the cells and the light particles preparation have been described previously[6]. Besides the light particles, we used two intermediate chromatophore fractions as a source of reaction center: "crude chromatophores" (that is, the pellet of the first 350000 $\times$ *g* centrifugation), and "purified chromatophores" (that is, chromatophores freed from ribosomes by CsCl treatment, then washed with 0.1 M phosphate buffer, pH 7.5).

Reaction center preparation

Two different fractionation procedures were used, each appropriate to a particular starting material.

(*1*) *Reaction center derived from light particles.* A light particle suspension in 0.1 M potassium phosphate buffer, pH 7.5 (3 mg protein per ml), was incubated with

stirring during 5 h at 5° with cetyltrimethylammonium bromide (CTAB) (final concentration, 0.3%, w/v). During incubation, part of the preparation aggregated. Fractions of this suspension (3 ml) were then layered on 45 ml of 0.6 M sucrose in SW-25-2 Spinco centrifuge tubes. After centrifugation for 1 h at 22000 rev./min, a pellet (devoid of raction center activity) was sedimented; a top brownish layer, in which activity was found, was recovered and freed from sucrose either by dialysis or Sephadex G-25 chromatography. At this step it was called "crude reaction center".

If necessary, further purification could be done by centrifugation at 350000 × *g* for 24 h. The supernatant phase was devoid of any photobleaching activity and contained about 70% of the initial protein in weight; all the activity was recovered in the pellet, which could not be redissolved without the use of a detergent. However, the nature of the detergent strongly influenced the stability of the preparation. In the presence of minimal amounts of CTAB (0.1%) or of sodium dodecyl sulfate, the activity was labile, whereas it was extremely stable in a non-ionic detergent. Therefore, a 0.1 M potassium phosphate buffer, pH 7.5, containing 0.15% Triton X-100 (w/v) was used for redissolving the pellet and for further studies of the purified reaction center.

(2) *Reaction center derived either from crude or from purified chromatophores.* These particles were suspended in 0.1 M potassium phosphate buffer, pH 7.5, and incubated for 1 h at 25° with CTAB (crude chromatophores, 8 mg protein per ml; purified chromatophores, 9.4 mg protein per ml; final CTAB concentration, 0.1%, w/v). The chromatophore suspensions were then layered on 0.6 M sucrose and centrifuged as described above. A similar top layer containing the activity, called "crude reaction center", was then recovered.

For further purification, addition of $(NH_4)_2SO_4$ at 5° to a final concentration of 70% (w/v) precipitated a component which contained all the reaction center activity. It was redissolved in a minimum amount of 0.1 M potassium phosphate buffer plus 0.15% Triton X-100 (w/v), pH 7.5, and dialysed against this buffer.

A final purification step employed gel filtration chromatography at 5° on a Sepharose 6B column (51 cm × 4 cm) equilibrated in 0.1 M potassium phosphate buffer, 0.15% Triton, 0.25 M NaCl, pH 7.5. Elution of reaction center samples (5–10 ml) was carried out at a flow rate of 48 ml/h with the same buffer; 2-ml fractions were collected and their spectra assayed. They were usually pooled into a number of fractions, which were concentrated to a small volume by ultrafiltration using an Aminco X-M 50 membrane.

Chemical analyses

Bacteriochlorophyll, protein and ubiquinone were determined as described previously[6].

P 865 content was determined spectroscopically from light *minus* dark difference spectra ($\Delta\varepsilon_{mM}$ (865 nm) = 93; see ref. 7).

Heme was determined on acetone–methanol (7:2, v/v) residue by the pyridine hemochrome method[8]. Separation of the *c* and *b* types was effected by HCl–acetone treatment. In the dithonite-reduced *minus* oxidized pyridine hemochrome difference spectrum, we used $\Delta\varepsilon_{mM}$ (550–575 nm) = 23 for *c*-type heme, and $\Delta\varepsilon_{mM}$ (556–537 nm) = 20.7 for *b*-type heme.

Iron was determined by atomic absorption spectroscopy on a Perkin–Elmer

Model 290. Standard solutions of iron (1–10 μg/ml) in 0.1 M phosphate buffer, pH 7.5, containing 0.15% Triton X-100 were used in each series of samples; control determinations with internal standards were performed.

Spectrophotometry

Spectra were recorded on a Model 14 R Cary spectrophotometer. IR-2 and IR-1 modes were used to record absolute spectra of active preparations either in light or dark conditions. For light *minus* dark differential spectra, the sample compartment was equipped with a unit at right angles to the measuring beam, composed of a 600-W Sylvania sun-gun lamp, lens and interference filter.

Redox potential measurements

The redox potential of reaction center preparations was determined spectrophotometrically by recording their absolute infrared spectra, in a redox buffer containing 0.15% Triton X-100, as a function of the potential. A special anaerobic cuvette designed after CUSANOVICH AND KAMEN[9] was used. The preparation was kept under nitrogen with intermittent bubbling, additions being made by injection through a septum. A couple of platinum and calomel electrodes (P 101, K 401; Radiometer) measured the potential of the sample continuously. They were standardized by comparison with a saturated quinhydrone solution. The titration began on a sample in $5 \cdot 10^{-2}$ M ferricyanide; small additions (5 μg) of reductant (0.33 M ferrocyanide or 1% dithionite) were made in order to decrease the potential gradually.

Analytical centrifugation

Centrifugation experiments were performed using a Beckman Model E ultracentrifuge equipped with absorption optics, with the assistance of Mrs. M. O. Mossé. To work selectively on an infrared absorption band of the reaction center, a tungsten lamp (Nachet, 30 W) was used instead of the visible source, and an appropriate interference filter (Balzers, 800 nm) was placed behind the lamp. Recording was done on infrared-sensitive plates (Kodak, spectroscopic Type 1N); they were measured on a Joyce–Loebl microdensitometer.

Sedimentation velocity determinations were done at 59 780 rev./min and 22°; solutions in 0.1 M potassium phosphate, 0.15% Triton, pH 7.5, were used, with an absorbance at 800 nm of around 0.8. Sedimentation equilibrium experiments were performed at 7747 rev./min; periods of at least 24 h were allowed in order to attain equilibrium. The partial specific volume of the reaction center particles was measured by comparing the sedimentation equilibrium of the preparation in 0.1 M potassium phosphate, 0.15% Triton, pH 7.5, buffer solutions containing either H_2O or ^2H_2O (Merck, Uvasol grade) according to the method of EDELSTEIN AND SCHACHMAN[10]; absorbance at 800 nm was 0.8 here, too. Densities of the two buffers were measured in a 5-ml pycnometer.

Molecular weight determination by gel filtration

The determination of the apparent molecular weight of the reaction center complex was done by gel filtration on a column (1.6 cm × 48 cm) of Sepharose 6B, in a 0.05 M Tris–HCl buffer, pH 7.5, containing 0.15% Triton X-100. The temperature was maintained at 5° and the flow rate was 4 ml/cm² per h; fractions of 1.5 ml were

collected. Several proteins of known molecular weight (cytochrome *c*, albumin, γ-globulin) were used to determine a calibration curve. Blue dextran was used as void volume marker.

RESULTS

Reaction center purification

CTAB proved to be effective in releasing a reaction center fraction from chromatophores at various stages of purification (crude or purified chromatophores, light particles). Only heavy particles appeared to release very little, if any, reaction center activity after incubation with CTAB. It may be noted that the amount of CTAB per g protein required for optimal reaction center recovery decreased when going from the crude chromatophores to the light particles.

Various chromatophore fractions were used to produce reaction centers in an effort to find the best experimental conditions. Criteria for purification were, on the one hand, the specific activity (P 865 content per g protein), and on the other, spectral characteristics (absence of light-harvesting bacteriochlorophyll, low level of contaminating pigments) (see below). The specific activities in the various purification procedures are given in Table I for some representative experiments, together with the yields expressed as moles P 865 recovered in the purified fraction, starting from 100 moles of bulk bacteriochlorophyll. It is obvious from Table I that the highest specific activities and the best yields were obtained when starting from low-iron light particles, and lower activities when starting from low-iron crude chromatophores. Light particles appeared then to be the best source material for purifying the reaction center. However, one may note that they did not lend themselves to large-scale production: their preparation required lengthy centrifugations and the final yield of reaction center was quite low. On the other hand, the method based on crude chromatophores was rapid and suitable for large-scale preparation, and was required for quantitative determination of iron in low-iron preparations (see below).

The purification of the light particle reaction center is based on centrifugation. The supernatant phase obtained by high-speed centrifugation was devoid of any activity and was found to contain solubilized proteins and several pigmented compounds tentatively identified from their absorption bands: carotenoids (absorbing

TABLE I

SPECIFIC ACTIVITIES AND YIELDS IN VARIOUS PURIFIED REACTION CENTER FRACTIONS

Mean values of *n* preparations.

	Light particles		*Purified chromatophores*		*Crude chromatophor*	
	Low-iron	*Normal*	*Low-iron*	*Normal*	*Low-iron*	*Normal*
P 865/protein (μmoles/g)	9.1 ($n = 6$)	5.5 ($n = 1$)	—	4.4 ($n = 2$)	1.3 ($n = 4$)	2.7 ($n = 3$)
P 865 recovered from 100 initial bacteriochlorophylls (mole/mole)	6.6 ($n = 6$)	3.4 ($n = 1$)		3.5 ($n = 3$)	1.96 ($n = 4$)	2.8 ($n = 3$)

at 490, 465 and 425 nm), bacteriopheophytin (535, 760 nm), a degradation product of bacteriochlorophyll (684 nm), and an unknown component strongly absorbing at 410 nm. Exactly the same result was obtained with $(NH_4)_2SO_4$ precipitation in the second procedure: here, too, the supernatant fluid was devoid of activity, contained solubilized proteins and had the same type of absorption spectrum.

Agarose chromatography generally did not greatly improve the specific activity; yet it allowed elimination of spurious components. The first eluted fraction contained reaction center particles contaminated with a small amount of the unbleachable bacteriochlorophyll absorbing at 840 nm. Then reaction center particles were eluted, followed by particles containing increasing amounts of the degradation product, and decreasing reaction center activity. The spectra and composition of these fractions will be discussed below.

Absorption spectra

The absorption spectrum of the reaction center preparation with the highest specific activity (that is, the purified fraction from light particles) is given in Fig. 1; it is strictly the same whether the preparation is derived from low-iron or from normal cells. The 865-, 802- and 595-nm bands may be assigned to bacteriochlorophyll, as well as the 365-nm maximum and possibly the 385-nm shoulder; the 758- and 535-nm bands have been attributed to bacteriopheophytin, and the 684-nm band to a decomposition product of bacteriochlorophyll. The 420-, 440- and 470-nm bands are tentatively assigned to residual carotenoids: spheroidene and spheroidenone have indeed been found in these preparations by thin-layer chromatography of pigment extracts (F. REISS-HUSSON AND G. JOLCHINE, unpublished results). The 285-nm band, mainly due to protein aromatic residues and possibly to pigments, has about the

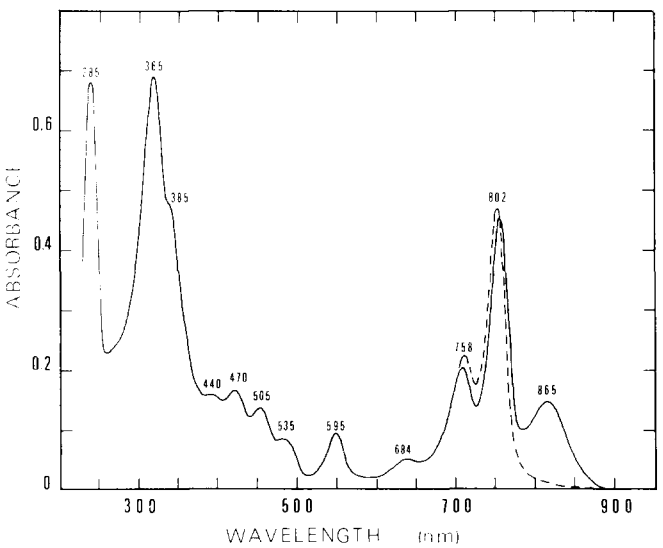

Fig. 1. Absorption spectrum of a purified reaction center from low-iron light particles. In the infrared range, the spectrum was recorded either under strong actinic light (– – – –) or in the dark (———).

same intensity as the one at 365 nm. The reproducibility of this spectrum over ten different preparations was excellent.

Purified reaction centers from purified chromatophores, obtained from low-iron or normal cells, exhibited spectra quite similar to those cited above. Minor variations were noted: as the specific activities were lower, the ratio $A_{865\ nm}/A_{365\ nm}$ decreased by a few per cent, a shoulder at 410 nm eventually appeared, and the absorption in the aromatic region (285 nm) increased. In contrast, large departures from the spectrum of Fig. 1 were obtained with crude or poorly purified reaction center preparations. The decrease in specific activity correlated with a decrease in the 802- and 865-nm bands and an increase in the visible range, particularly at 684 and 410 nm.

The infrared absorption changes brought about by illumination of the reaction center are a shift towards the blue of the 805-nm band, and bleaching of the one at 865 nm (Fig. 1). This bleaching was total in all purified reaction center fractions, and reversible in the dark after a few minutes. These changes are not exactly identical to those seen in the chromatophores. Light *minus* dark spectra of particles exhibited a blue shift of the 805-nm band, and two minima at 842 and 880 nm, respectively. The relative amplitudes of these two minima were found to vary with culture conditions: the 880-nm minimum was more pronounced in low-iron particles (one may note that the B 870 bulk bacteriochlorophyll increased relatively to the B 855 component in low-iron particles[6]). The 842-nm minimum could be due to a red shift of the B 855 component. In any case, invariable infrared absolute and difference spectra were obtained for the various reaction center preparations, in spite of these variations.

Redox potential

The redox potential of the reaction center was determined by titrating in the dark the reduction of a preparation previously completely oxidized, the reduction being measured by the absorbance in the 865-nm band. It was rather surprising to find that a 2 μM P 865 solution could not be bleached completely by a 1 mM ferricyanide solution, and that at least a 0.01 M ferro–ferricyanide buffer was required to conveniently cover the oxidized range. In such a redox buffer, the titration followed the theoretical law for a one-electron change with a midpoint potential $E_m = 525$ mV at pH 7.5 and 22°. In a control experiment, it was checked that, in a chromatophore suspension, the midpoint potential of the light induced change at 800 nm was 445 mV, a value in good agreement with previous data on other organisms[11]. Thus it appears that the reaction center when isolated by our methods had a redox potential significantly more oxidizing than *in vivo*.

Monodisperse character and particle weight determination

A number of analytical centrifugation experiments were performed in order to check the homogeneity of the purified fractions as well as to determine their particle weight. Optical absorption at 800 nm was used in order to selectively detect the particles bearing the reaction center, and to exclude any contaminating material still present. With such a setting, all purified fractions behaved as a single component, as judged from sedimentation velocity experiments (Fig. 2), at least in the very low concentration range used ($A_{800\ nm} < 1$). The sedimentation coefficients of various purified reaction centers are reported in Table II. Depending on the origin of the reaction centers, they varied in the range of 3.4–4.0 S. No marked influence of source

material was apparent. Consequently, their particle weights did not vary appreciably.

The surprisingly monodisperse character of the reaction center particles led us to determine the partial specific volume and the particle weight from sedimentation equilibrium experiments in H_2O and ^2H_2O buffers. This was done on a purified reaction center preparation extracted from purified normal chromatophores. As infrared absorption optics were required for its selectivity, and no scanning system was available to us, photographic recording was used. The darkening of the plates was measured as microdensitometer deflection. A linear relation was found between the logarithm of this deflection and r^2, (r being the distance to the rotor axis) (Fig. 3). On the basis of this observation, assuming that the deflection was proportional to the absorbance at 800 nm, *i.e.* to the reaction center concentration, we derived the partial specific volume and the particle weight from the slopes of the plots relative to the H_2O and ^2H_2O buffers, according to the method of EDELSTEIN AND SCHACHMAN[10]. The ratio of the particle weight in the deuterated to that in the non-deuterated solvent was taken as 1.0155, a mean value for a variety of proteins. The partial specific volume of the reaction center particle thus determined was 0.877 cm^3/g (at 22°), and the particle weight was 153000.

It should be noted that infrared absorption optics were a prerequisite for these

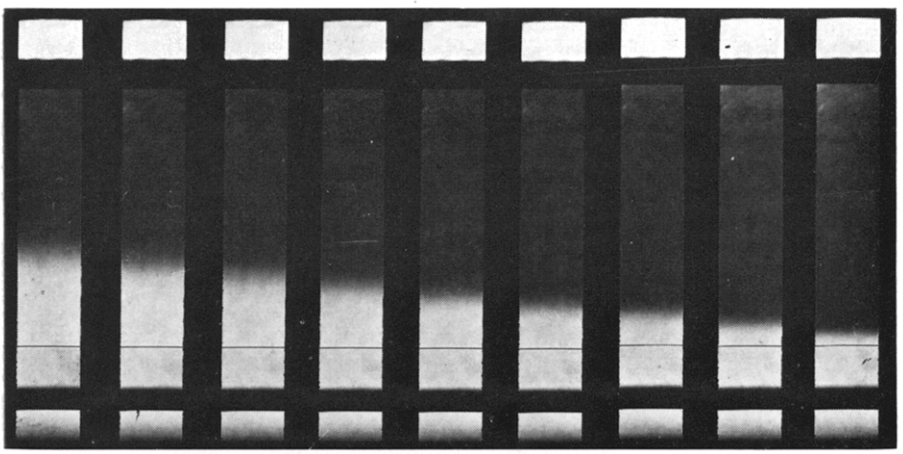

Fig. 2. Sedimentation velocity experiment, followed by absorption at 800 nm, on a purified reaction center from low-iron light particles in 0.1 M potassium phosphate, 0.15% Triton X-100 (w/v), pH 7.5, at 22° ($A_{800\ nm} = 0.8$). 59780 rev./min, 8-min intervals.

TABLE II

SEDIMENTATION COEFFICIENTS OF VARIOUS PURIFIED REACTION CENTERS

Values given in S, at 22°, for reaction center in 0.1 M potassium phosphate 0.15% Triton X-100, pH 7.5 (approx. 2.5 μM P 865). Mean values of n experiments.

Origin of reaction center	s (S)	n
Crude chromatophores, low-iron	3.8	1
Crude chromatophores, normal	3.9	1
Purified chromatophores, normal	3.4	2
Light particles, normal	4.0	4

experiments. Indeed, some of the preparations revealed two components when absorbance in the visible range (*e.g.* 365 nm) was used, and even three components were found with the Schlieren method (F. REISS-HUSSON AND G. JOLCHINE, unpublished results).

An independent determination of the particle weight was done by gel chromatography. Purified reaction centers (extracted from light particles or from purified chromatophores) were assayed on a Sepharose column calibrated with proteins of known molecular weight; all experiments were performed in the same buffer containing 0.15 % Triton X-100 (w/v). The reaction center particles were eluted as a single symmetrical peak; a value of 150000 (± 10 %) was thus obtained for the particle weight.

Reaction center composition

Purified reaction centers from crude chromatophores (low-iron and normal) were analysed for some possible electron carriers (ubiquinone, heme and non-heme iron) (Table III). For comparison, data relative to crude chromatophores are also given.

The purified reaction centers contained a high amount of ubiquinone, whatever the iron supply; non-heme and *c*-type heme were also present. No *b*-type heme was apparent, from the pyridine hemochromes as well as from low temperature reduced *minus* oxidized difference spectra(I. AGALIDIS *et al.*, unpublished results). When compared to the chromatophores from which they were derived, normal reaction centers contained as much non-heme iron, and more *c*-type heme; in contrast, in low-iron

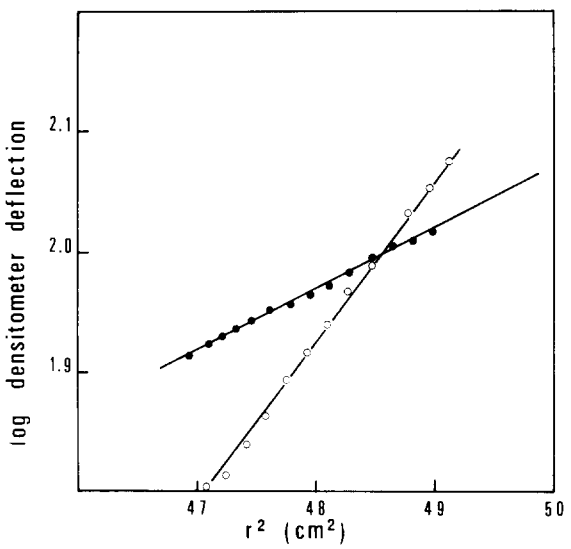

Fig. 3. Sedimentation equilibrium analysis of a purified reaction center in 0.15 % Triton X-100, 0.1 M potassium phosphate buffer, pH 7.5, prepared either in H_2O (○—○) or ^2H_2O (●—●) ($A_{800\ nm}$ = 0.8), at 22°. Centrifugation was performed at 7447 rev./min, with 800 nm light and photographic recording on infrared-sensitive plates. These were scanned on a Joyce–Loebl microdensitometer; the logarithm of the deflection was plotted as a function of the square of the distance from the axis of rotation.

TABLE III

UBIQUINONE, HEME AND NON-HEME IRON CONTENTS OF CRUDE CHROMATOPHORES AND PURIFIED REACTION CENTERS PREPARED THEREFROM

Mean values of three independent preparations, expressed in moles.

	Chromatophores		*Reaction centers*	
	Low-iron	*Normal*	*Low-iron*	*Normal*
P 865	1*	1*	1*	1*
Ubiquinone	10.2	8.4	8.6	8.0
c-type heme	0.2	0.45	0.12	1.5
b-type heme	0.015	0.15	0	0
Non-heme iron**	0.59	0.60	0.21	0.50

* 1 mole P 865 per 100 bulk bacteriochlorophylls was assumed on the basis of the light-induced changes.

** Calculated as the difference between total iron and heme iron contents.

reaction centers the *c*-type heme was decreased by half, the non-heme iron by one-third. When the two types of reaction centers were compared, iron deficiency brought about a slight decrease in non-heme iron and a more pronounced fall in *c*-type heme; the molar ratios of these two components to P 865 fell to far below 1.

In several experiments, analyses were performed not only on the purified reaction centers but also on the various Agarose fractions. It was found that all fractions contained ubiquinone and heme iron. Yet heme iron was mostly eluted in the fractions which had a high specific activity, whereas ubiquinone and non-heme iron were eluted mostly in the last fractions which contained the smallest particles rich in degradation pigments and of a very low activity. At this level of purification, it cannot be stated that the measured ubiquinone and non-heme iron are characteristic of the reaction center particles, and the values of Table II are to be taken as maximal.

DISCUSSION

In our experiments, CTAB released from various chromatophore fractions (except from the heavy particles) a reaction center with a unique absorption spectrum and a size always in the same range (*s* approx. 4 S; mol.wt. approx. 150000). Yet the specific activity of these reaction center fractions (P 865 per protein weight) could increase by a factor of 4 when light particles, which were the most purified chromatophores, were used instead of crude chromatophores. The simplest explanation is that CTAB releases the reaction center from any chromatophore fraction as a 4-S particle. At the same time, inactive parts of the membrane are solubilised. Those particles which are of the same size as the reaction center are selected in the purification steps and contribute to the decrease in specific activity.

The character of the reaction center does not vary using cells in two different metabolic states, and three types of chromatophore fractions. It may be noted here that in the case of the blue-green mutant of *Rps. spheroides*, Triton X-100 releases a reaction center bound to much larger particles (mol. wt. approx. 650000), with a specific activity comparable to that of the fraction extracted, in our case, from normal

crude chromatophores[12]. Such a difference in particle size may be attributed either to the variation in detergent nature, or to differences in membrane structure, or both.

The unique absorption spectrum observed in our work bears striking similarities to that published for the reaction centers of carotenoid-less mutants of *Rps. spheroides* (R-26)[12] and of *Rps. rubrum* (G9)[2]. Of course, in our case residual carotenoids are present; one notes also an increase in the ratios A_{865nm}/A_{365nm} and A_{365nm}/A_{280nm}, due to higher specific activity. Apart from these variations, the positions of the bands are the same. In all cases, 2 P 800 molecules appear to be present for 1 P 865. Such similarity may be taken as indirect proof for the "native" state of the bacteriochlorophyll complex in the isolated reaction center, and for the identity of structure of this complex among various purple non-sulfur bacteria. Such a "native" state, based only on spectroscopic criteria, does not mean that some other properties of the reaction center cannot be modified due to its isolation in the presence of detergent. The redox potential, going from 445 mV *in vivo* to 525 mV *in vitro* may be such a property.

Besides the photoactive bacteriochlorophylls, at least two other pigments are present in the reaction centers prepared here. The carotenoids seem to be less easily eliminated than the bulk bacteriochlorophyll, suggesting perhaps a tighter link between them and the reaction center. Bacteriopheophytin is the other pigment: its amount seems to be the same in this case and in *Rps. spheroides* R-26[12], as well as in *Rps. rubrum* G9[2]. If this bacteriopheophytin were only a degradation product, one would not expect such a constant production within three strains, and two types of detergent.

The partial specific volume of the particle bearing the reaction center, 0.88 cm^3/g, is indicative of a lipoprotein rather than a protein. Analyses of the total lipid and bound detergent are now in progress, to determine their contribution to the particle weight.

The possible electron transport components detected in the reaction center include heme, non-heme iron, and ubiquinone. The *b*-type heme is completely absent, which is at variance with the case of *Rps. spheroides* R-26[12]; its absence is in better agreement with its location in the electron transport chain, which is quite remote from the reaction center bacteriochlorophyll. On the contrary, *c*-type heme is present as expected, even in low-iron conditions. It is not known if it belongs to only one hemoprotein, or to two cytochromes, the later being the case for *Rps. viridis* and *Chromatium* Fraction A[13]. In low-iron conditions, the molar ratio between the *c*-type heme and P 865 decreases from 1.5:1 to 0.12:1, whereas non-heme iron is not so drastically affected, yet being very low, 0.2 atom per P 865 molecule. This amount, which has to be taken as a maximal value (see above) may be compared to the values given[12] for *Rps. spheroides* R-26 reaction center, 13.2 and 4.3 non-heme iron atoms per 1 P 865, respectively, for two preparations differing in the Triton X-100 concentration used for incubation. In our case, such low values are certainly connected with the smaller size of the reaction center particles. From our results one may conclude that even in iron-deficient preparations, reaction centers still contain *c*-type heme and non-heme but at such low concentrations that these compounds might not a play role as electron donor or acceptor, nor be intimately associated with bacteriochlorophyll.

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