Biochimica et Biophysica Acta, 501 (1978) 470—477

BBA 47460

PROPERTIES OF PHOTOCHEMICAL REACTION CENTERS PURIFIED FROM *RHODOPSEUDOMONAS GELATINOSA*

BETTY J. CLAYTON and RODERICK K. CLAYTON
Division of Biological Sciences, Cornell University, Ithaca, N.Y. 14853 (U.S.A.)
(Received August 1st, 1977)

Summary

Reaction centers were isolated from a carotenoidless mutant of *Rhodopseudomonas gelatinosa* by hydroxyapatite chromatography of purified chromatophores treated with lauryl dimethyl amine oxide. Absorption spectra and spectra of light-induced absorbance changes are similar to those of reaction centers from *Rhodopseudomonas sphaeroides*. The ratio of absorbance at 280 nm to that at 799 nm was 1.8 in the purest preparations. The extinction coefficient at the 799 nm absorption maximum was estimated to be 305 ± 20 $mM^{-1} \cdot cm^{-1}$. The molecular weight based on protein and chromophore assays was found to be $1.5 \cdot 10^5$; the reaction center protein accounted for 6% of the total membrane protein. These reaction centers contained no cytochrome and showed just two components of apparent molecular weights 33 000 and 25 000 in polyacrylamide gel electrophoresis. The chromatophores contained 42 molecules of antenna bacteriochlorophyll for each reaction center.

Introduction

The photochemical reaction centers isolated from *Rhodopseudomonas sphaeroides*, *Rhodospirillum rubrum* and *Chromatium vinosum* all have similar optical absorption spectra and light-induced difference spectra. The protein components of these reaction centers as revealed by polyacrylamide gel electrophoresis are also similar; three polypeptides in the approximate range 20 000—30 000 daltons, although the reaction centers come from highly diverse organisms [1—6].

We have prepared purified reaction centers from *Rhodopseudomonas gelatinosa*, and find strong similarities to the foregoing types in terms of optical properties, but conspicuous differences in the protein components.

Abbreviation: LDAO, lauryl dimethyl amine oxide.

Methods

Cultures and chromatophore preparation. Rp. gelatinosa strain TG-9 was isolated from local mud; our identification of this species was confirmed by Dr. Norbert Pfennig. Mutant phenotypes were obtained by exposing cells of strain TG-9 to 0.03% aqueous ethylmethansulfonate in 0.01 M potassium phosphate buffer, pH 7.0 (hereafter called phosphate buffer). After 5 min exposure in the illuminated growth cabinet followed by 40 min in the dark, the cell suspension was diluted 10-fold into 5% aqueous $Na_2S_2O_3$ and then serially into 0.01 M phosphate buffer. Selected dilutions were spread onto culture plates (1.5% agar in tap water with 0.5% yeast extract and 0.3% casein hydrolysate). The plates, incubated aerobically at 30°C in the dark, yielded some colonies showing abnormal pigmentation. A blue-green (carotenoidless) mutant strain designated EM-1 was selected for the work described here. Absorption spectra of cells or chromatophores of the mutant showed no detectable colored carotenoids. Attempts to purify reaction centers from the wild type strain TG-9 were not successful; the best preparations remained contaminated by antenna pigments. Cultures of strain TG-9/EM-1 were grown anaerobically in the light; the harvested cells were kept frozen until used for the isolation of chromatophores (intracytoplasmic membrane fragments) and reaction centers.

Purified chromatophores were prepared as described earlier [7] for *Rp. sphaeroides*, except that in the density gradient centrifugation the material was layered onto a three-step discontinuous sucrose gradient: 0.6, 1.3 and 1.6 M sucrose. After centrifugation about 70% of the pigmented material was collected just below the 0.6/1.3 M sucrose interface; a lesser and more turbid fraction was taken just above the 1.3/1.6 M interface. The less dense chromatophores yielded reaction centers of greater purity. These purified chromatophores were diluted and recentrifuged to remove sucrose, and suspended in 0.01 M Tris · HCl, pH 7.5 (hereafter called Tris buffer) to an absorbance of 50 at the long wave absorption peak of bacteriochlorophyll near 865 nm.

Isolation of reaction centers. 40 ml of the purified chromatophores were mixed with 4.5 ml of 30% aqueous lauryl dimethyl amine oxide (LDAO). After 5 min the mixture was placed onto a column of 100 ml hydroxyapatite that had been flushed with 0.01 M phosphate buffer containing 0.05% LDAO. The system was eluted with 0.01 M phosphate buffer + 0.05% LDAO until the eluate was nearly colorless (about 200 ml). Elution was continued with a linear gradient, 0.01—0.3 M phosphate buffer with 0.05% LDAO, total volume 600 ml. The flow rate was kept below 4 ml/min. Reaction centers began to emerge at about 0.15 M phosphate; the earliest fractions were of the highest purity. Later fractions showed increasing contamination by heme proteins and antenna bacteriochlorophyll (B860). The material collected between about 0.15 and 0.2 M phosphate was pooled and dialyzed overnight against 0.01 M Tris buffer with 0.05% LDAO. The dialyzed material was made 1% in LDAO and passed into a 40 ml hydroxyapatite column prepared as before. The elution program was as before except that the volumes and flow rate were 0.4 of the former. Selected fractions were dialyzed overnight against 0.01 M Tris buffer with 0.05% LDAO and then concentrated by membrane filtration ("Minicon

Concentrator", Amicon Corp., Lexington, Mass. 02173). This procedure was the residue of many less successful attempts in which chromatography on diethylaminoethyl cellulose and ammonium sulfate fractionation were explored in conjunction with the use of hydroxyapatite.

Hydroxyapatite was prepared by the method of Siegelman et al. [8]. All steps from the addition of LDAO to purified chromatophores were done at about 4°C with minimal exposure to light, and excessive exposure to high concentrations of LDAO was avoided. Without these precautions the reaction centers deteriorated rapidly. The purified reaction centers, in 0.01 M Tris buffer with 0.05% LDAO, were stable for many hours at 4°C and for days when frozen and stored at −20°C.

Analytical procedures. Polyacrylamide gel electrophoresis with sodium dodecyl sulfate was done by the modified method of Laemmli [9] described earlier [10]; polypeptides were dissociated by exposure to 1.5% sodium dodecyl sulfate and 5% mercaptoethanol at 65°C for 30 min. As markers we used bovine serum albumin (68 000 daltons), equine cytochrome *c* (12 400 daltons), and reaction centers from *Rp. sphaeroides* (27 500, 22 500 and 20 000 daltons) for the polypeptides H, M and L, respectively; an average of our earlier findings [10] and those of Feher [2]. The stained gels were scanned optically at 550 nm with a home-made scanner; basically a split-beam spectrophotometer. The distance from the origin to the ion front was bout 8 cm.

Protein was estimated by the method of Lowry et al. [11].

Optical absorption spectra and difference spectra were measured with a Cary 14R spectrophotometer fitted for cross-illumination of the sample; the spectra could be stored and subtracted from each other with a TN-1500 Signal Averager (Tracor-Northern, Inc., Middleton, WI 53562). A home-made amplifier provided an interface between the Cary 14R and TN-1500 [12]. For measurements at low temperature the samples were suspended in 80% glycerol and mounted in the optical head of a closed-cycle helium refrigerator ("Spectrim"; Cryogenic Technologies Inc., Waltham, Mass. 02154) as described elsewhere [13].

Cytochrome (predominantly a *c*-type with α band at 552 nm) in the reaction center preparations was assayed by the optical absorption difference at 552 nm between the oxidized and reduced forms, assuming (by analogy with other cytochromes of the *c* type) a differential extinction coefficient of 20 $mM^{-1} \cdot cm^{-1}$. Potassium ferricyanide was used for oxidation and sodium dithionite for reduction.

The sum of bacteriochlorophyll and bacteriopheophytin was estimated by extracting one volume of material (chromatophores or reaction centers) with 20 volumes of acetone/methanol (7 : 2, v/v), acidifying the extract with 1/50 volume 5 M HCl to convert bacteriochlorophyll to bacteriopheophytin, and measuring the absorbance at the maximum near 525 nm. The extinction coefficient of bacteriopheophytin in 7 acetone/2 methanol was taken to be 23.5 $mM^{-1} \cdot cm^{-1}$ [14].

Results

Optical properties

The reaction center fractions that emerged earliest from the hydroxyapatite column were the purest; an absorption spectrum of such a fraction is shown in Fig. 1. The pattern of absorption by bacteriochlorophyll and bacteriopheophytin from 500 to 1000 nm resembles closely that of reaction centers from *Rp. sphaeroides*, except that the two longest wave peaks come at slightly shorter wavelengths (799 and 850 nm, compared to 802 and 860—865 nm for *Rp. sphaeroides*). The position of the 850 nm band was labile; in some preparations it was closer to 860 nm, and in cells of either the wild type or mutant strain TG-9/EM-1 the light-induced bleaching of this band was near 870 nm. From the similarity of spectra we infer that the reaction center of *Rp. gelatinosa*, like that of *Rp. sphaeroides*, has four molecules of bacteriochlorophyll and two of bacteriopheophytin [15], the latter responsible for the peaks at 535 and 754 nm. At low temperature the band at 535 nm is resolved into two at 529 and 545 nm, and the long wave band is shifted to 880 nm. The light-induced difference spectrum is also like that of *Rp. sphaeroides* but with features at slightly shorter wavelengths: negative peaks at 807 and 850 nm, and positive ones at 785 and 1245 nm. In chromatophores of *Rp. gelatinosa* the longer wave negative peak is at 870 nm (*P*-870).

Absolute molar extinction coefficients of the reaction centers were determined by measuring absorption spectra of samples and then extracting them with acetone/methanol to determine their content of bacteriochlorophyll plus bacteriopheophytin. We assumed that each reaction center contains four bacteriochlorophyll and two bacteriopheophytin, so that the molarity of reaction centers is one-sixth that of tetrapyrrole in a given sample. Results are shown in Table I. We estimated the precision of these determinations to be ±7%.

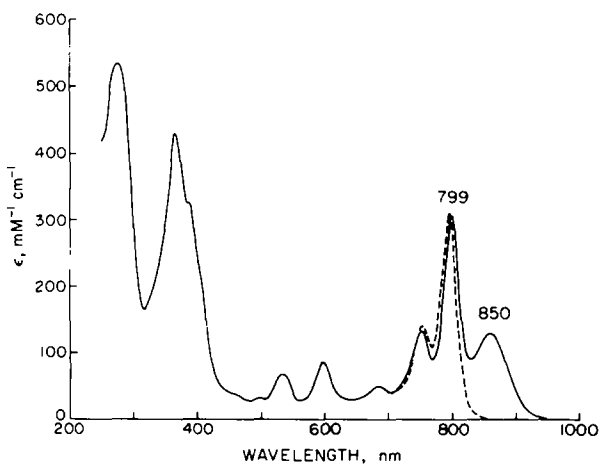

Fig. 1. Absorption spectra of reaction centers purified from *Rp. gelatinosa*, strain TG-9/EM-1. Solid curve, sample in weak monochromatic light during measurement. Dashed curve, sample exposed to strong white light (No. 2 IR mode of the Cary 14R spectrophotometer) during measurement. The ordinate has been normalized to show the absolute extinction coefficient; see the text and Table I.

TABLE I

DETERMINATION OF ABSOLUTE MOLAR EXTINCTION COEFFICIENTS

Reaction centers were extracted with acetone/methanol and the absorbance at 525 nm of bacteriopheophytin in the acidified extract was compared with peak absrobances of the reaction centers. The extracts were 20-fold dilutions of the reaction center samples. We assumed that one reaction center yields six molecules of bacteriopheophytin in the acidified extract, arising from four bacteriochlorophyll and two bacteriopheophytin (see the text). The molar concentration of bacteriopheophytin in the extract must then be multiplied by 20/6 to give the molar concentration of reaction centers in the original sample.

	Acidified acetone/methanol extract		Reaction centers before extraction				
	A_{525nm}	μM Bacteriopheophytin (μM)	μM reaction centers; (20/6) · μM bacteriopheophytin in extract	Peak absorbances (1 cm path)		ϵ(mM^{-1} · cm^{-1}) ($a_{1\cdot cm}$/mM)	
Rp. gelatinosa				a_{799nm}	a_{850nm}	ϵ_{799}	ϵ_{850}
	0.0545	2.3	7.7	2.48	1.07	320	139
	0.061	2.6	8.7	2.52	1.06	290	122
Mean						305	130
Rp. sphaeroides				a_{802nm}	a_{865nm}	a_{802}	ϵ_{865}
	0.053	2.25	7.5	2.24	1.00	300	133
	0.054	2.3	7.7	2.21	0.96	287	124
Mean						294	128

Concurrent measurements with reaction centers from *Rp. sphaeroides* gave values in good agreement with those reported earlier [15].

Protein components

In our purest reaction center preparations from *Rp. gelatinosa* the ratio of absorbance at 280 nm to that at 799 nm was 1.8. 1 mol of such reaction centers contained 150 kg of protein by the method of Lowry et al. [11]. There was less than 0.1 cytochrome per reaction center.

Serial fractions from the hydroxyapatite column contained progressively more cytochrome 552 *, rising to more than two per reaction center. Another substance, possibly a cytochromoid, was nearly absent in the earliest fractions but appeared in increasing amounts in later fractions. This substance was abundant in a preparation of chromatophores that had been treated as follows **: Chromatophores with 1%LDAO were centrifuged over a sucrose gradient containing 0.3% Triton X-100 and 1 M urea at pH 10. The lightest fraction was extracted with nine volumes of acetone at −10°C and the residue was solubilized with 0.01 M Tris buffer containing 1% LDAO. Absorption spectra of the solubilized material showed a Soret peak at 407 nm, negligible α-band structure, and no evidence of reaction centers. Upon addition of sodium dithionite the Soret band shifted to 409 nm and was attenuated by about 40%;

* This is probably the same as the low potential *c*-type cytochrome with an α peak at 548 nm at 77 K, as described by Dutton [16].

** This treatment is similar to the procedure described by Tiede et al. [17] for the isolation of reaction centers from *Chr. vinosum*.

no α-band structure appeared. We known nothing about the origin or possible function of this substance, which appears to be far more abundant than cytochrome 552 in LDAO-treated chromatophores. We shall call it C407.

Polyacrylamide gel electrophoretograms of the purest reaction center preparations from *Rp. gelatinosa* showed just two major components, of apparent molecular weights 33 000 and 25 000 (Fig. 2, trace 3). If other components were present, they either did not enter the gel or did not bind the stain. Acetone extraction of the samples prior to electrophoresis did not affect the results. There is no simple and unique way to combine the 33 000 and 25 000 dalton components so as to give the molecular weight of 150 000 that was indicated by the protein analysis.

Traces 4 and 5 of Fig. 2 show gel scans of one of the less pure reaction center fractions, which contained C407 and about one cytochrome 552 per reaction center. The sample of trace 4 contained albumin as a marker, and that of trace 5 was marked with reaction centers from *Rp. sphaeroides*, giving the bands labeled H, M and L. These electrophoretograms show bands A (42 000 daltons), D (21 000 daltons) and E (16 000 daltons) as well as the bands B and C of the more pure reaction center sample seen in trace 3. Bands A and E became more pronounced in samples with more C407 and cytochrome 552, but we lack sufficient data to identify these components unequivocally.

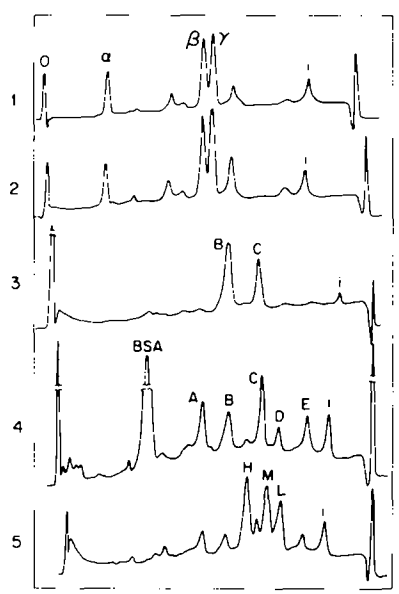

Fig. 2. Scans of polyacrylamide gels showing electrophoresis of dissociated proteins from *Rp. gelatinosa*. The origin is indicated by o and the leading edge (ion front) by i. Trace 1, purified chromatophores from wild type strain TG-9. Trace 2, purified chromatophores from carotenoidless mutant strain TG-9/EM-1. The apparent molecular weights of components α, β and γ, determined from other gels containing markers (see text), are 87 000, 34 000 and 31 000. Trace 3, purified reaction centers from strain TG-9/EM-1. Components B and C have apparent molecular weights 33 000 and 25 000. Traces 4 and 5, crude reaction centers from strain TG-9/EM-1. Added markers were bovine serum albumin (BSA) in trace 4 and *Rp. sphaeroides* reaction centers (giving peptides H, M and L) in trace 5. The apparent molecular weights of components A, B, C, D and E are 42 000, 33 000, 25 000, 21 000 and 16 000, respectively.

Traces 1 and 2 are for purified chromatophores of *Rp. gelatinosa*, wild type strain TG-9 and mutant strain TG-6/EM-1, respectively. These show major polypeptides (α, β and γ) of apparent molecular weights 87 000, 34 000 and 31 000; the band just to the right of γ has the same apparent molecular weight (25 000) as component C in scan 3 (note, from the position of the ion front i, that scans 1 and 2 are more compressed than scan 3). These apparent molecular weights were determined from similar gels which contained albumin, equine cytochrome *c* and *Rp. sphaeroides* reactions centers as markers.

Purified chromatophores from the mutant strain TG-9/EM-1 were analyzed for their content of protein, bacteriochlorophyll and reaction centers; the assay of reaction centers was based on the magnitude of the reversible bleaching at 870 nm induced by saturating illumination. We found $2.5 \cdot 10^6$ g of protein per mol of reaction centers in these chromatophores. If we take the molecular weight of purified reaction centers to be $1.5 \cdot 10^5$, we estimate that reaction centers contribute 6% of the total membrane protein. These assays also showed the presence of 42 molecules of antenna bacteriochlorophyll for each reaction center in the chromatophore, and gave an extinction coefficient of 152 $mM^{-1} \cdot cm^{-1}$ at 865 nm for the antenna bacteriochlorophyll in situ.

Discussion

In chromatophores of photosynthetic bacteria, the primary photochemistry is followed by the transfer of an electron from a cytochrome of the *c* type to oxidized bacteriochlorophyll in the reaction center. *Rp. gelatinosa* resembles *Rp. viridis* and *Chr. vinosum* in that this electron transfer occurs rapidly (within milliseconds or less) at liquid nitrogen temperature [18]. In contrast, the electron transfer from cytochrome to oxidized bacteriochlorophyll at liquid nitrogen temperature is too slow to measure in chromatophores from *Rp. sphaeroides*, *Rp. capsulata* and *Rs. rubrum* [18]. Rapid electron transfer from cytochrome to oxidized bacteriochlorophyll at low temperature is correlated with firmness of binding of the cytochrome to the chromatophore membrane. When cells of *Rp. sphaeroides*, *Rp. capsulata* or *Rs. rubrum* are broken, the *c*-type cytochrome that interacts directly with reaction ceners is released in soluble form unless it is trapped within the membrane-bound vesicles (see ref. 19). In *Rp. gelatinosa*, *Rp. viridis* and *Chr. vinosum* such cytochromes remain bound to the intracytoplasmic membrane. But chromatophores of *Rp. gelatinosa*, treated with LDAO, yield reaction centers free of cytochrome, whereas those of *Rp. viridis* and *Chr. vinosum* do not [6,20]. Thus *Rp. gelatinosa* differs from the other species in the manner in which its *c*-type cytochromes are held in the membrane.

The reaction centers of *Rp. sphaeroides*, *Rs. rubrum* and *Chr. vinosum*, three widely divergent species of photosynthetic bacteria, have nearly identical absorption spectra and protein components. Reaction centers from *Rp. gelatinosa* resemble the others in their absorption spectrum, but their protein components appear to be quite different and they are far less stable in isolated form. If we accept current taxonomic structures, we can speculate that after the genus *Rhodopseudomonas* had become consolidated, viable variations in the protein matrix of the reaction center began to emerge. *Rp. gelatinosa* may

thus prove useful in comparative studies of the architectures of photosynthetic membranes.

The electron paramagnetic properties of reaction centers and chromatophores of *Rp. gelatinosa* have been studied in collaboration with Drs. P.L. Dutton and R.C. Prince, and will be described in a separate communication.

Acknowledgements

This work was supported by Contract No. E(11-1)-3162 with the U.S. Energy Research and Development Administration and Grant No. PCM76-01556 from the National Science Foundation.

References

1 Clayton, R.K. and Wang, R.T. (1971) Methods Enzymol. 23, 696—704
2 Feher, G. (1971) Photochem. Photobiol. 14, 373—387
3 Wang, R.T. and Clayton, R.K. (1973) Photochem. Photobiol. 17, 57—61
4 Noël, H., van der Rest, M. and Gingras, G. (1972) Biochim. Biophys. Acta 275, 219—230
5 Feher, G. and Okamura, M.Y. (1976) Brookhaven Symp. Biol. 28, 183—194
6 Lin, L. and Thornber, J.P. (1975) Photochem. Photobiol. 22, 37—40
7 Clayton, R.K. and Clayton, B.J. (1972) Biochim. Biophys. Acta 283, 492—504
8 Siegelman, H.W., Wieczorek, G.A. and Turner, B.C. (1965) Anal. Biochem. 13, 402—404
9 Laemmli, U.K. (1970) Nature 227, 680—685
10 Clayton, R.K. and Haselkorn, R. (1972) J. Mol. Biol. 68, 97—105
11 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
12 Vermeglio, A. and Clayton, R.K. (1976) Biochim. Biophys. Acta 449, 500—515
13 Clayton, R.K. (1977) in Photosynthetic Organelles, Special Issue of Plant and Cell Physiol., Japan, No. 3 (Miyachi, S., Katoh, S., Fujita, Y. and Shibata, K., eds.), pp. 87—96. Center for Academic Publications, Japan
14 Straley, S.C. (1972) Ph.D. Thesis, Cornell University, Ithaca, N.Y. 14853, U.S.A.
15 Straley, S.C., Parson, W.W., Mauzerall, D.C. and Clayton, R.K. (1973) Biochim. Biophys. Acta 305, 597—609
16 Dutton, P.L. (1971) Biochim. Biophys. Acta 226, 63—80
17 Tiede, D.M., Prince, R.C. and Dutton, P.L. (1976) Biochim. Biophys. Acta 449, 447—467
18 Kihara, T. and Chance, B. (1969) Biochim. Biophys. Acta 189, 116—124
19 Dutton, P.L. and Prince, R.C. (1977) in the Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), Plenum Publishing Co., New York, in the press
20 Pucheu, N.L., Kerber, N.L. and Garcia, A.F. (1976) Arch. Microbiol. 109, 301—305