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Photochemical Systems of *Rhodospirillum rubrum*. Light-Induced Reactions and Biological Functions of *c*-Type Cytochromes in Relation to P-870†

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ABSTRACT: Evidence from light-induced reactions, electrofocusing, amino acid analysis, and immunochemical characterization is presented to prove that cytochrome c_2 and cytochrome C-422 of *Rhodospirillum rubrum* are identical. The designation cytochrome c_2 also applies to the high-potential cytochromes of cyclic photochemical electron transport in *Rhodopseudomonas spheroides*, *Rhodopseudomonas viridis*, and *Rhodopseudomonas capsulata*, all of which can substitute for *R. rubrum* cytochrome c_2 by donating electrons to P-870 of *R. rubrum*. From light-induced reactions it is also suggested that cytochrome c' and the low-potential cytochrome C-428 of *R. rubrum* may be identical but no biological function can be assigned to this hemoprotein based on our studies. Only one photochemical system could be found to operate in *R. rubrum*, and this system appeared to be capable of oxidizing both the high-potential cytochrome c_2 and added cytochrome c'. However, no reaction of P-870 with the native cytochrome C-428 could be observed.

This study was undertaken with the aim to correlate the membrane-bound *c*-type cytochromes, C-422 and C-428 (Sybesma and Fowler, 1968), implicated in secondary electron-transport reactions in *Rhodospirillum rubrum*, with the chemically characterized, soluble cytochromes, c_2 and c', of this organism (Dus *et al.*, 1968; Kamen *et al.*, 1971; Kennel *et al.*, 1973). Furthermore, we intended to clarify the position of these cytochromes within the photochemical electron-transfer chain relative to the corresponding reaction center units and to scrutinize the concept of the possible existence of more than one type of reaction center in *R. rubrum* (Sybesma and Fowler, 1968; Fowler and Sybesma, 1970).

Previously, two photochemical systems were suggested to function in whole cells of *R. rubrum* (Sybesma and Fowler, 1968) based on different action spectra for the light-induced visible and near infrared absorbancy changes under continuous (Sybesma, 1969) and flashing (Sybesma and Kok, 1969) illumination. This was in agreement with action spectra of cytochromes C-555 and C-552 obtained by Morita (1968) in whole cells of *Chromatium vinosum* strain D which indicated oxidation of these cytochromes by different reaction centers. Measurements of the light-induced absorbancy changes as a function of redox potential in cells of *C. vinosum* strain D (Cusanovich *et al.*, 1968) and of *R. rubrum* (Fowler and Sybesma, 1970) led to similar conclusions. Specifically, in the experiments with *R. rubrum*, only one system, involving oxidation of cytochrome C-422 and interaction with the typical P-870 containing reaction center, seemed to operate under high-potential conditions. At potentials near zero, however, C-428, the low-potential cytochrome, was found to operate predominantly and the concomitant absorbancy changes in the near-infrared region were no longer those indicative of the typical P-870 containing reaction center. Among other changes, in this case, an increase of absorbancy was observed at 905 nm. A similar increase at 905 nm under low potential conditions had been found previously in *C. vinosum* strain D (Cusanovich *et al.*, 1968). This observation was taken to indicate the presence of a second reaction center component, P', thought to be associated with noncyclic photochemical electron transport and capable of oxidizing only the low-potential cytochrome. An alternate explanation was offered by Vredenberg *et al.* (1965) who suggested that these absorbancy changes may reflect a conformational change in the chromatophore membrane.

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More recently, however, reaction center preparations obtained from both *Rhodopseudomonas viridis* (Thornber *et al.*, 1969; Case *et al.*, 1970) and *C. vinosum* strain D (Thornber, 1970; Parson and Case, 1970) were shown to contain one high- and one low-potential cytochrome each, and it was demonstrated that oxidation of both types of cytochromes was mediated by only one photochemical system. In the present paper we report the detailed studies leading to functional identification and characterization of c-type cytochromes present in reaction center preparations of *R. rubrum*, and clarifying their participation in light-induced reactions. These investigations have been extended, in part, to *Rhodopseudomonas spheroides*, *Rps. viridis*, and *Rhodopseudomonas capsulata*. In addition, we describe experiments designed to obtain evidence for the postulated second reaction center component, P′. Preliminary accounts of some of these findings have been given previously (Sybesma *et al.*, 1972; Smith and Dus, 1972).

Experimental Section

Materials. $Na^{125}I$ was purchased from New England Nuclear Corp., and spectrally pure Chloramine-T was obtained from Eastman Organic Chemicals. Ampholyte solutions for isoelectric focusing were purchased from LKB-Produkter AB, Bromma, Sweden. All other chemicals were of reagent grade and were used without further purification except for sodium dodecyl sulfate which was purchased from Fisher Scientific Co. It was recrystallized from 95% ethanol before use.

Cultivation of Microorganisms. Cells of three species of non-sulfur, facultative photoheterotrophs were grown as follows: *R. rubrum* (Giesbrecht, strain 4) on the completely synthetic medium of Ormerod *et al.* (1961); *Rps. spheroides* (van Niel, 2.4.1) on medium A of Sistrom (1960) using yeast extract; *Rps. viridis* (NHTC 133) on the medium of Eimhjellen *et al.* (1963) using yeast extract and succinate. All cultures were grown routinely for 6–7 days at ambient temperatures of 31 ± 1.5° in 13-l. carboys using circular, fluorescent light banks. The cells were harvested by means of a Sharples centrifuge, and either used immediately or stored frozen at 0°.

Experimental Procedures. Chromatophores and reaction centers of these organisms were prepared from freshly harvested cells as described previously (Smith *et al.*, 1972) but in order to ensure isolation of active reaction center preparations from frozen cells it was found necessary to lower the sodium dodecyl sulfate concentration from 5.4 to 5.0 g per mmol of BChl.[1] Specifically, preparations referred to, throughout this paper, as "large reaction centers" had a molecular weight of ~100,000 daltons and contained P-870 and cytochrome c_2 in equimolar quantities. In addition, cytochrome c' was found in variable amounts and several small, unidentified proteins were also present in molar quantities roughly equivalent to the amount of P-870. Spirilloxanthin and ubiquinone were found in ratios to P-870 of 3:1 and 1.7:1, respectively. The amount of BChl in reaction center preparations was determined using the extinction coefficients, ϵ_{mM} of 75 mM^{-1} cm^{-1} at 770 nm (*in vitro*) and $\Delta\epsilon$ of 93 mM^{-1} cm^{-1} at 865 nm (*in vivo*), given by Clayton (1966) and Bolton *et al.* (1969), respectively. For calculation of cytochrome contents of reaction center preparations we used the $\Delta\epsilon_{mM}$ (reduced minus oxidized) values of 100 mM^{-1} cm^{-1} at 422 nm (Olson and Chance, 1960) and 23 mM^{-1} cm^{-1} at 551 nm (Kakuno *et al.*, 1971).

Light-induced absorbancy changes were measured either with a split-beam difference spectrophotometer at 1°, using cuvets of 1-mm light path (Sybesma and Fowler, 1968), or with a Cary 14R recording spectrophotometer, equipped with an actinic light source (650-W bulb) for cross-illumination of a 1-cm sample cuvet. Absorption spectra were traced with the Cary recording spectrophotometer, Model 14R. In order to monitor light-induced absorbancy changes of the reaction center preparations as a function of redox potential, the redox couples described by Cusanovich *et al.* (1968) were employed. These titrations were carried out with a 1% solution of sodium dithionate in 10 mM NaOH and involved redox potential poising at known values before activation.

Electrofocusing experiments were performed in a combined density (sucrose 0–50%) and pH (3–10) gradient using apparatus and techniques outlined by Vesterberg and Svensson (1966). Commercial ampholyte solutions were employed at a concentration of 1% v/v. Electrolyses were carried out for 48–72 hr until stable minimal current was attained at a total power output of less than 1 W. A Gilford Model 2000 spectrophotometer equipped with 1-cm light-path flow cells was used to monitor the column effluent after electrofocusing.

Cytochromes c_2 and c' were isolated from the supernatant resulting from sonication of the cells and centrifugation as well as from the large reaction center preparation by electrofocusing and subsequent dialysis and electrodialysis against 50 mM phosphate (pH 6.5). Cytochromes c_2 were also purified from *R. rubrum*, *Rps. spheroides*, *Rps. viridis*, and *Rps. capsulata* by the procedure of Kamen *et al.* (1963).

Amino acid analyses were carried out on a modified Beckman-Spinco Model 120 amino acid analyzer, using the stepwise four-buffer elution program of Dus *et al.* (1966). Protein samples were hydrolyzed with 5.9 N HCl in sealed-glass tubes for 24 and 48 hr at 105–110°. Oxidation of methionine and decomposition of tyrosine and tryptophan were almost completely prevented by addition of thioglycolic acid (final concentration 0.6%, v/v) to the HCl. Cysteine and cystine were measured as cysteic acid after performic acid oxidation and subsequent acid hydrolysis for 24 hr of the protein sample (Hirs, 1956).

Anti-*R. rubrum* cytochrome c_2 rabbit antibodies were obtained by injecting 5 mg of pure cytochrome c_2, prepared by the method of Kamen *et al.* (1963), emulsified with complete Freunds adjuvant, into the footpads of an adult albino rabbit on day 0, and again after 24 days. Blood samples of approximately 10 ml were collected three times starting 2 weeks after the booster shot. All antisera used exhibited antibody titers in excess of 1 mg/ml. Cytochrome c_2 of *R. rubrum* was labeled with ^{125}I using the procedure of London *et al.* (1967).

The inhibition experiments were carried out according to the procedure of Atassi and Saplin (1968) except that it was modified for use with labeled antigen to increase the sensitivity. For quantitative inhibition, aliquots of 50 μl of unlabeled antigen were placed in 200 μl of 0.15 M NaCl–50 mM sodium phosphate buffer (pH 7.7) and mixed with 50 μl of antiserum (1:5 dilution). The mixture was incubated at room temperature for 2 hr before an aliquot of labeled antigen, sufficient to saturate the antibody at equivalence, was added in 50 μl of the NaCl–phosphate buffer. After incubation for 48 hr at 10°, the precipitates were spun down at 3000*g*, washed once with cold buffer, and then dissolved in 200 μl of 0.2 N NaOH. The dissolved material was added to 15 ml of scintillation fluid, made from 3 l. of toluene, 2.25 l. of ethanol, and 24 g of 2,5-di-

[1] Abbreviation used is: BChl, bacteriochlorophyll.

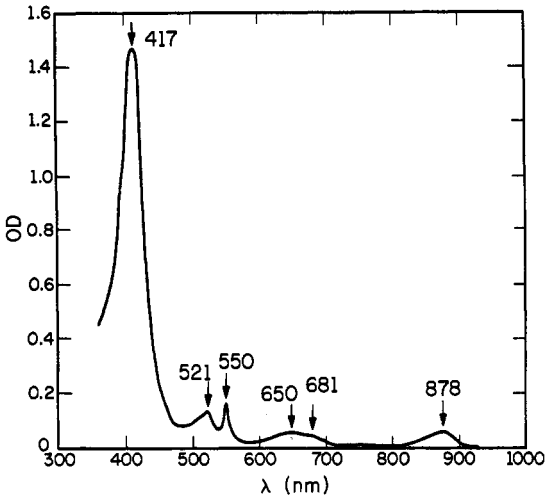

FIGURE 1: Absorption spectrum of supernatant fraction 2 of *R. rubrum*. After sonication in 50 mM Tris-HCl (pH 8.0), the cell debris was removed by centrifugation (15,000*g* for 20 min). The supernatant was obtained by further centrifugation (144,000*g*) of the "crude" chromatophores for 4 hr at 4°. This supernatant was passed through a column of Sephadex G-100 (2 × 60 cm) equilibrated with 10^{-4} M NH_4OH, and the fraction containing material <50,000 daltons was lyophilized. This material was redissolved in a small volume of 10^{-3} M NH_4OH. The spectrum was traced with a Cary 14R recording spectrophotometer.

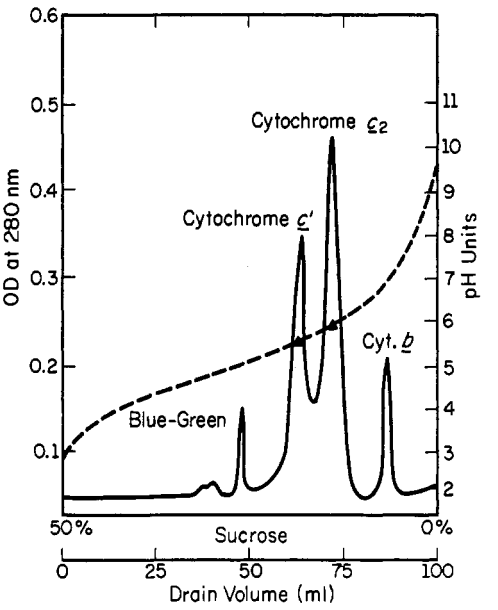

FIGURE 2: Electrofocusing of supernatant fraction 2 of *R. rubrum*. The lyophilized protein fraction was dissolved in 2 ml of 10^{-3} M NH_4OH and added in two equal aliquots to tubes 10 and 11 of the sucrose gradient. After electrolysis for 50 hr the column was drained and the effluent was monitored for absorbancy at 280 nm with a Gilford spectrophotometer, Model 2000, using a flow-through cuvet of 10-mm light path.

phenyloxazole, and counted for 5 min with a Beckman liquid scintillation counter, Model LS-30, using the ^{14}C window. Each inhibition experiment was carried out in triplicate. A Digital PDP-8 computer and program were used to determine mean inhibitions and standard deviations.

Results

Identification and Functional Characterization of Cytochromes. The low molecular weight (<50,000) fraction of the supernatant obtained by centrifuging of the "crude" chromatophores at 144,000*g* and subsequent chromatography of the supernatant on a column of Sephadex G-100 seemed rich in cytochromes as judged by the absorption spectrum (see Figure 1).

This observation was confirmed by separation of the soluble hemoproteins of this fraction by electrofocusing. As Figure 2 demonstrates, cytochromes c_2 and c' are the predominant heme-bearing proteins of the supernatant; in fact, based on spectral identification and quantitation, they are present in amounts to account for approximately 80% of the total of these cytochromes in intact cells. It follows then that they can be bound to the membrane structure only loosely since the conditions used for preparation of chromatophores rendered them freely soluble.

Isoelectric points of 5.9 and 5.4 were measured at 22° for cytochrome c_2 and cytochrome c', respectively. These values are in excellent agreement with the values recorded for these proteins in the literature (Sletten and Kamen, 1968) and identical with those obtained for the soluble hemoproteins resolved by electrofocusing of the large reaction center preparations (Smith *et al.*, 1972). The minor component found to have a p*I* of 6.9 may well be cytochrome *b* (Kakuno *et al.*, 1971) while the blue-green band of p*I* approximately 5 could be a trace of solubilized bacteriochlorophyll protein. Owing to the small amounts found in these experiments, however, no attempts at positive identification of the minor components were made. The separation pattern produced by electrofocusing of cytochromes present in reaction center preparations (Smith *et al.*, 1972) was identical with the pattern seen in Figure 2 except that in the former much less cytochrome c' relative to the amount of cytochrome c_2 was observed, and not even a trace of the minor band tentatively designated as cytochrome *b* was found. The bands containing cytochromes c_2 and c' were collected, freed from sucrose by dialysis, and subsequently electrodialyzed against 50 mM phosphate (pH 6.5) for complete removal of ampholytes, and lyophilized.

A light-minus-dark difference spectrum obtained after addition of the supernatant to a suspension of chromatophores of *R. rubrum* in 50 mM Tris-HCl buffer (pH 8.0) is shown in Figure 3A. The troughs centering at 422, 522, and 551 nm are identical with those resulting from light-induced oxidation of C-422 (Sybesma and Fowler, 1968; Fowler and Sybesma, 1970). These experiments were repeated using cytochrome c_2 isolated by electrofocusing from the supernatant instead of the whole supernatant. The same difference spectra were obtained, affording a first strong indication that cytochromes c_2 and C-422 might be identical. Mammalian cytochrome *c* was able to substitute for cytochromes c_2 in this reaction but reacted at significantly slower rates.

No spectral indication of C-428 oxidation was obtained under these conditions, even at low light intensities (less than 0.6 nEinstein/(cm^2 sec)). As shown in Figure 3B, a light-minus-dark difference spectrum with troughs centering at 432 and 561 nm was observed, however, after allowing the redox environment to become lower by stoppering the cuvet in order to provide more anaerobic conditions (after approximately 5 min). This observation suggested light-induced oxidation of cytochrome c' since an identical oxidized-minus-reduced difference spectrum was produced with a chemically oxidized sample of purified cytochrome c'. A ratio of nearly 12 for γ to α peaks was found for cytochrome c' which is

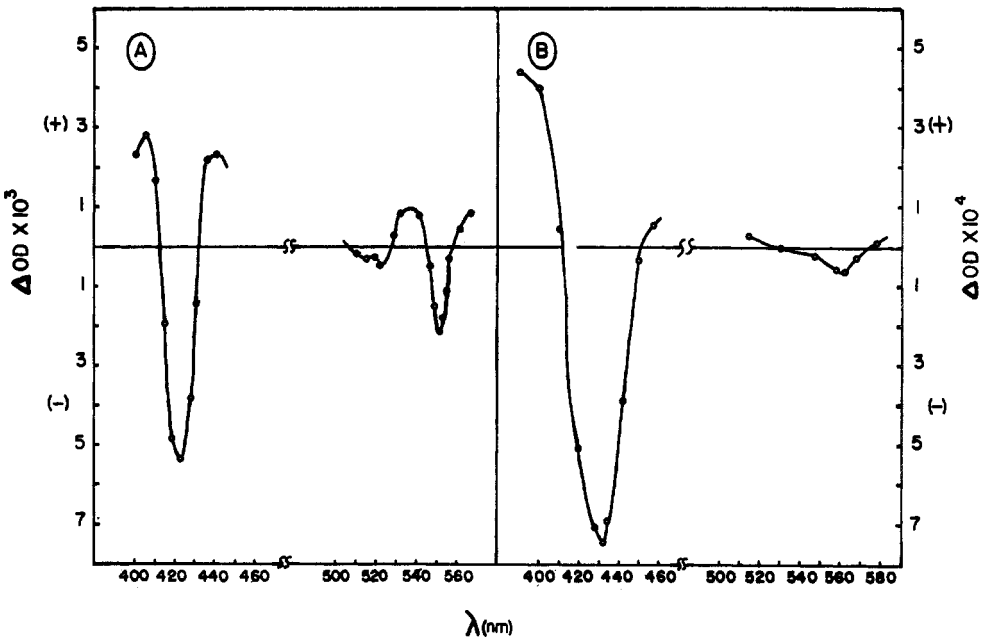

FIGURE 3: Light-minus-dark difference spectra of *R. rubrum* chromatophores: (A) after addition of the 144,000*g* supernatant to a suspension of chromatophores in 50 mM Tris-HCl (pH 8.0); (B) same as under part A after 15 min in the dark. All measurements were made at 1° in a split-beam difference spectrophotometer. Actinic illumination at 865 nm was used at an intensity of 3.1 nEinsteins/(cm² sec).

close to that of 10 reported for C-428 by Parson (1967). Moreover, identical spectral properties were obtained upon addition of a purified preparation of cytochrome *c'* to the reaction center preparation of *R. rubrum*. Thus, the typical P-870 found in the reaction center of *R. rubrum* is capable of oxidizing low-potential cytochromes such as cytochrome *c'*.

From kinetic experiments (Figure 4) it can be seen that at cytochrome c_2 concentrations of 0.5 mol/mol of P-870, during the light-on phase, absorbancy changes at 422 nm indicate a rapid initial change in the positive direction due to an overcompensating P-435 change in the region of 400–450 nm (Parson, 1967). This is followed by a rapid decrease indicative of C-422 oxidation. Upon turning off the light, immediately an absorbancy decrease of P-435 and an absorbancy increase of C-422 typical of the light-off phases for both components are observed. At higher concentrations of cytochrome c_2 only changes indicative of C-422 oxidation are seen while lower concentrations reflect only positive absorbancy changes typical of P-435.

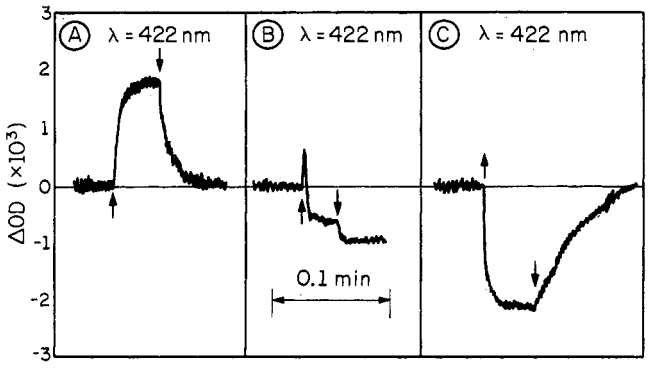

FIGURE 4: Light-induced absorbancy changes of *R. rubrum* chromatophores at 422 nm: (A) before addition of cytochrome c_2; (B) after addition of purified cytochrome c_2 (0.5 mol/mol of P-870); (C) after addition of purified cytochrome c_2 (>0.5 mol/mol of P-870). Upward and downward pointing arrows reflect light-on and light-off transitions, respectively. All measurements were made at 1° in a split-beam difference spectrophotometer. Actinic illumination at 865 nm was used at an intensity of 3.1 nEinsteins/(cm² sec).

Table I compares the amino acid compositions of the cytochromes c_2 isolated from electrofocusing experiments both on the supernatant (preparation 1) and on reaction center preparations of *R. rubrum* (preparation 2) to the composition of cytochrome c_2 used to establish the amino acid sequence (Dus *et al.*, 1968). The correlation of these compositions is sufficiently close to permit positive identification of these hemoproteins. We therefore conclude that the high-potential cytochrome *c* of the supernatant and cytochrome C-422 of our large reaction center preparations are identical with cytochrome c_2. Thus it is this hemoprotein which functions in cyclic photochemical electron transport by donating electrons to light-activated P-870. This conclusion is further supported by immunochemical evidence given in Figure 5. Using radioimmunoassays identical inhibition of binding (100%) between ^{125}I-labeled cytochrome c_2 of *R. rubrum* and anti-*R. rubrum* cytochrome c_2 antibodies was observed for all three cytochrome preparations. The same studies also revealed a strong cross-reaction (75%) between free cytochrome c_2 and cytochrome c_2 bound in the large reaction center preparation of *R. rubrum*. On the other hand, very little cross-reaction (about 10%) was found between these antisera and horse heart cytochrome *c* although this hemoprotein can, at least to some extent, substitute for cytochrome c_2 in the light-induced oxidation reaction. It should be noted that cytochrome C-422, as part of the reaction center complex, probably contained sodium dodecyl sulfate to the extent of 0.55 g/g of dry weight as was found for the total complex (Smith *et al.*, 1972) while all of the sodium dodecyl sulfate was removed during isolation of the cytochrome by electrofocusing.

In order to extend this functional characteristic of cytochrome c_2 of *R. rubrum* to similar high-potential cytochromes found in other photosynthetic bacteria, we compared preparations of these cytochromes obtained by electrofocusing of supernatants and corresponding reaction center preparations

TABLE I: Comparison of Amino Acid Composition of Cytochrome c_2 and Related Cytochrome *c* Preparations of *Rhodospirillum rubrum*.[a]

Amino Acids	Cryst Cyt c_2	Prep 1	Prep 2
Cysteic acid	1.8	1.6	1.5
Aspartic acid	12.9	13.3	13.5
Methionine sulfone	1.8	1.9	1.3
Threonine	7.8	8.1	7.5
Serine	4.9	5.5	5.8
Glutamic acid	10.0	9.4	9.6
Proline	3.0	2.9	2.8
Glycine	8.1	8.5	8.2
Alanine	15.1	14.2	14.6
Valine	6.0	5.4	5.2
Isoleucine	2.0	1.6	1.8
Leucine	7.9	7.6	8.4
Tyrosine	4.9	4.1	3.9
Phenylalanine	5.0	5.2	5.5
Histidine	2.0	1.6	1.9
Lysine	16.9	16.1	16.5
Tryptophan	0.8	0.5	0.7
Arginine	0.0	0.3	0.1
Total	112		

[a] Preparations 1 and 2 refer to cytochrome *c* isolated by electrofocusing from the supernatant cytochrome fraction and from the large reaction center preparation, respectively. The values represent the average of three analyses. Cysteic acid and methionine sulfone were determined from performate oxidized samples. The values of tyrosine and tryptophan refer to hydrolyses with 6 N HCl to which 0.6% thioglycolic acid was added before use. The values for crystalline cytochrome c_2 are taken from Dus *et al.* (1968).

in two other non-sulfur photoanaerobes selected for their decreasing ability to produce cytochrome *c'*.

Figure 6A,B represents the traces resulting from monitoring the column effluents of electrofocusing experiments on preparations of *Rps. spheroides* and *Rps. viridis*, respectively. Cytochrome c_2 is present in both organisms in large amounts but only a trace of cytochrome *c'* is found in the supernatant of *Rps. spheroides*, and apparently none at all is present in *Rps. viridis*. The latter organism instead contains another low-potential cytochrome *c* (Olson and Clayton, 1966). The predominant hemoprotein in the supernatant of *Rps. spheroides* is the spheroides heme protein (SHP), an atypical cytochrome described by Meyer *et al.* (1973).

Light-induced oxidations of cytochromes c_2 of *Rps. spheroides*, *Rps. capsulata*, and *Rps. viridis* using a large reaction center preparation of *R. rubrum* from which the cytochrome c_2 had been completely removed by passage through a column of Sephadex G-100 (2 × 60 cm) showed in all cases a linear increase of moles of cytochrome c_2 oxidized per mole of P-870 to moles of the respective cytochrome c_2 added to the solution. Thus all four cytochromes c_2 are able to substitute for one another in this reaction; they also show closely similar reaction rates and efficiencies.

One or Two Photochemical Systems in R. rubrum. Despite our determined efforts to find and isolate a second reaction center component (P') our search for this postulated complex was unsuccessful. Because of our excellent yields of large

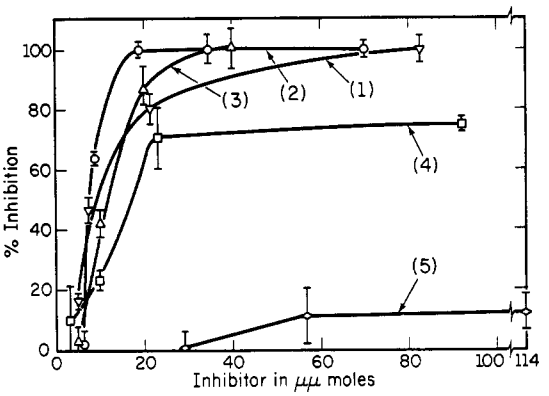

FIGURE 5: Radioimmunoassay of inhibition of antigen–antibody binding between ^{125}I-labeled cytochrome c_2 and anti-cytochrome c_2 antisera. Rabbit antisera against *R. rubrum* cytochrome c_2 were incubated for 2 hr at room temperature with increasing amounts of competing, unlabeled antigen in NaCl–phosphate buffer (pH 7.7). Aliquots of ^{125}I-labeled antigen, sufficient to saturate the antibody at equivalence, were then added in the same buffer and incubated for 48 hr at 10°. The precipitates were first spun down at 3000*g* and washed once with cold buffer, then dissolved in 0.2 N NaOH, added to scintillation fluid and counted for 5 min in a Beckman liquid scintillation counter, Model LS-30, using the ^{14}C window. All experiments were carried out in triplicate: (1) crystalline cytochrome c_2 of *R. rubrum*, prepared according to Kamen *et al.* (1963); (2) cytochrome *c* isolated by electrofocusing from the cytochrome fraction of the *R. rubrum* supernatant; (3) cytochrome *c* isolated by electrofocusing from large reaction center of *R. rubrum;* (4) large reaction center preparation of *R. rubrum*; (5) horse heart cytochrome *c*.

reaction centers, we suspected that a second photochemically active complex might possibly be contained in these reaction center preparations. However, titrations of light-induced absorbancy changes in various redox buffers (Cusanovich *et al.*, 1968) failed to reveal any increases either at 870 or 905 nm. A check of the absorbancy changes in the visible region indicated that absorbancy changes due to C-422 decreased commensurate with those of P-870. No absorbancy changes due to cytochrome *c'* were seen and the ratio of change at 428 nm relative to that at 422 nm remained unaltered. The lack

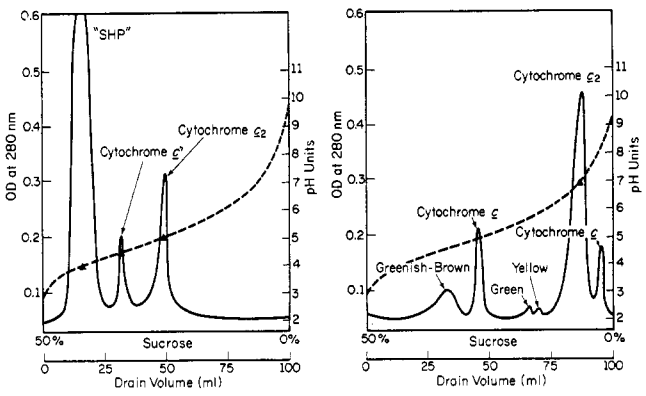

FIGURE 6: Electrofocusing of cytochrome fractions: (A, left) supernatant fraction 2 of *Rps. spheroides;* (B, right) supernatant fraction 2 of *Rps. viridis*. The cytochrome fractions were obtained by passing the corresponding 144,000*g* supernatant of the "crude" chromatophores through a column of Sephadex G-100 equilibrated with 10^{-4} M NH_4OH. The lyophilized low molecular weight fraction (<50,000 daltons) was redissolved in 2 ml of 10^{-3} M NH_4OH and added in two 1-ml aliquots to tubes 10 and 11 of the sucrose gradient. After electrolysis for 45 hr the columns were drained and the effluents were monitored at 280 nm with a Gilford spectrophotometer, Model 2000, using a flow-through cuvet of 1-cm light path.

of a change corresponding to cytochrome *c'* at the lower potentials could be attributed, however, to the fact that this component is present in the reaction center in rather small quantities (Sybesma *et al.*, 1972; Smith *et al.*, 1972). The purified reaction center preparation obtained after gel filtration on Sephadex G-100, which removes the cytochromes, still showed the positive absorbancy changes attributable to P-435. The kinetics and light saturation of the 435-nm change are similar to those at 602 and 870 nm which are due to reaction center bacteriochlorophyll.

Discussion

Light-induced absorbancy changes of *c*-type cytochromes had been observed in whole cells of *R. rubrum* both by Duysens (1957) and by Sybesma (1967), yet no such changes could be found in chromatophore preparations. Although chemical titrations with ferricyanide provided evidence that a certain amount of these cytochromes was still present in the chromatophores (Fowler and Sybesma, 1970), it is now apparent, in the light of our electrofocusing experiments, that the bulk of the hemoproteins (80%) was removed from the chromatophores with the last supernatant of the purification procedure. The remaining 20% cannot be seen in chromatophores of *R. rubrum* because the light-induced absorbancy changes of the cytochromes, especially those of cytochrome C-422, are obscured by larger positive absorbancy changes in the region between 400 and 450 nm due to P-435. Kinetic and light saturation studies of the 435-nm absorbancy increase relative to the decrease at 870 nm with the reaction center preparations of *R. rubrum* suggest that at least in these preparations P-435 may well reflect reaction center bacteriochlorophyll changes. Similar conclusions were reached by Seibert and DeVault (1971) working with chromatophores of *C. vinosum* strain D.

Addition of exogenous cytochrome c_2 to chromatophores, either in the form of purified hemoprotein or in the mixture of proteins present in the 144,000*g* supernatant, permits one to demonstrate light-induced cytochrome reactions typical of C-422. This observation also proves that in *R. rubrum* the *c*-type soluble cytochromes and the membrane-bound *c*-type cytochromes are identical. No *c*-type cytochromes other than c_2 and *c'* have been found in this organism during the present investigation. In contrast, several *c*-type cytochromes in addition to cytochrome c_2, and even several cytochromes *c'*, were isolated from *Rps. capsulata* (L. Turner and K. Dus, in preparation). Similarly, a variety of *c*-type cytochromes exists in *C. vinosum* strain D (Kennel and Kamen, 1971), 80% of them membrane bound; but in this organism the soluble cytochromes seem to differ from the membrane-bound hemoproteins. Thus it is not surprising that light-induced cytochrome reactions can be readily followed in chromatophores of *C. vinosum* strain D (Kennel and Kamen, 1971) and in *Rhodopseudomonas gelatinosa* (Dutton, 1971) where electrofocusing experiments with the supernatant revealed only traces of cytochromes (K. Dus *et al.*, unpublished observations). Horio and Yamashita (1963) and Bartsch (1963) had previously suggested that cytochrome c_2 may function as the primary electron donor to oxidized P-870 in *R. rubrum* but the experiments reported in this paper provide conclusive proof that cytochrome c_2, with a redox potential of approximately +340 mV at pH 7.0 (Kamen and Vernon, 1955) and a p*I* of 5.9, is identical with C-422 which undergoes light-induced oxidation and chemical oxidation at high redox potentials in n tact cells, chromatophores, and reaction center preparations of *R. rubrum*. It was of interest to demonstrate that this concept also applies to other photosynthetic bacteria that contain cytochrome c_2, *Rps. spheroides*, *Rps. viridis*, and *Rps. capsulata*, and that these cytochromes are indeed functionally interchangeable. Moreover, they are structurally homologous as evidenced by immunochemical cross-reactions (W. J. Litchfield and K. Dus, in preparation), sequence homology (K. Dus *et al.*, unpublished results), and nuclear magnetic resonance spectroscopy (Krejcarek *et al.*, 1971). In *Rps. viridis* it had previously been shown by Olson and Clayton (1966) that cytochrome C-558, which we now find to be closely related to cytochromes c_2, donates electrons to P-960. It is intriguing to see that such great similarity exists between the high potential cytochromes of organisms which apparently employ reaction centers of quite different characteristics, and even different bacteriochlorophyll.

Our experiments also provide suggestive evidence that cytochrome *c'* which was found in small and variable quantities in the reaction center preparations of *R. rubrum* may be identical with the low-potential cytochrome C-428 known to undergo light-induced oxidation in intact cells of this organism. The biological function of this hemoprotein, however, is not yet clear. From data obtained in this investigation, and from earlier indications (Thornber *et al.*, 1969), it is apparent that *Rps. viridis* contains no cytochrome *c'*. Instead, another low-potential cytochrome *c* was found to be associated with the reaction center (Thornber *et al.*, 1969). Both the low- and the high-potential cytochromes *c* of *Rps. viridis* were found in the soluble fraction. Surprisingly, this is not the case in *C. vinosum* strain D where large quantities of cytochrome *c'* are present in the supernatant, yet a different low-potential cytochrome *c* is found associated with the reaction center (Thornber, 1970; Kennel and Kamen, 1971). In conclusion, it seems that although there is no obligatory requirement for the presence of a CO-binding hemoprotein for the light-induced low-potential reaction in photosynthetic bacteria, cytochrome *c'* may function in this capacity in some species.

The absence of an identifiable P'-type reaction in the reaction center preparation of *R. rubrum*, coupled with the fact that these preparations were made especially from old cells which supposedly should contain exclusively the second photochemical system (Govindjee and Sybesma, 1971), supports the concept that only one photochemical system operates in *R. rubrum;* this system may be capable of oxidizing both low- and high-potential c-type cytochromes, although no reaction of the native cytochrome C-428 with reaction center bacteriochlorophyll could be demonstrated. The possibility that two light reactions may occur in the single photochemical reaction center (R. Govindjee and C. Sybesma, unpublished observations; W. R. Smith, Jr., and R. Govindjee, in preparation) is not ruled out by the evidence presented in this paper.

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