Proc. Natl. Acad. Sci. USA
Vol. 77, No. 1, pp. 87–91, January 1980
Biochemistry

Isolation and characterization of the pigment–protein complexes of *Rhodopseudomonas sphaeroides* by lithium dodecyl sulfate/polyacrylamide gel electrophoresis

(light-harvesting bacteriochlorophyll/reaction centers/carotenoids/absorption spectra/fluorescence emission spectra)

RICHARD M. BROGLIE*, C. NEIL HUNTER*, PHILIPPE DELEPELAIRE†, ROBERT A. NIEDERMAN*, NAM-HAI CHUA†, AND RODERICK K. CLAYTON‡

*Department of Microbiology, Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey 08903; †The Rockefeller University, New York, New York 10021; and ‡Section of Botany, Genetics and Development, Plant Science Building, Cornell University, Ithaca, New York 14850

Contributed by Roderick K. Clayton, September 6, 1979

ABSTRACT **When purified photosynthetic membranes from *Rhodopseudomonas sphaeroides* were treated with lithium dodecyl sulfate and subjected to polyacrylamide gel electrophoresis at 4°C, up to 11 pigment–protein complexes were resolved. Absorption spectra revealed that the smallest complex contained reaction center pigments and the others contained the antenna components B850 and B875 in various proportions. Of these antenna complexes, the largest was almost entirely B850 and the smallest contained only B875. After solubilization at 100°C and electrophoresis on polyacrylamide gradient gels, the B850 complex gave rise to two polypeptide components migrating with apparent M_r of 10,000 and 8000, whereas with the B875 complex, two components were observed with apparent M_r of 12,000 and 8000. The reaction center complex gave rise to only the 24 and 21 kilodalton polypeptide subunits. Fluorescence emission spectra showed maxima at 872 and 902 nm for B850 and B875, respectively. Analyses of bacteriochlorophyll *a* and carotenoids indicated that, in the B875 complex, two molecules of each of these pigments are associated with the two polypeptides. The associations of B850 and B875 in large and small complexes obtained by lithium dodecyl sulfate treatment are consistent with models of their organization within the membrane.**

The photosynthetic apparatus of the photoheterotrophic bacterium *Rhodopseudomonas sphaeroides* is localized in a system of intracytoplasmic membranes (1) which, upon mechanical disruption, gives rise to vesicles termed "chromatophores" (2). These contain most of the antenna (light-harvesting) and reaction center bacteriochlorophyll *a* (Bchl)–protein complexes (2). Radiant energy harvested by antenna migrates to the reaction center where productive photochemistry occurs (3, 4). Absorption spectra reveal two antenna complexes designated B850 and B875 on the basis of their absorption maxima in the near-infrared (5). B875 is associated with the reaction center in a molar ratio of about 30:1 (6), unlike B850 levels which vary with changes in light intensity (6) or the stage of membrane development (7). A detailed understanding of these Bchl–protein complexes requires their isolation from the membrane. After detergent treatment, reaction center preparations have been purified from chromatophores of both the carotenoidless mutant R-26 (8, 9) and the wild type (10); similarly, the B850 complex has also been isolated from the latter (11). To our knowledge, no reports on the isolation of B875 from wild-type *R. sphaeroides* have appeared.

Recently, a novel procedure has been developed for the isolation of chlorophyll–protein complexes from thylakoid membranes of *Chlamydomonas reinhardtii* and higher plants (12). After solubilization of membranes in lithium dodecyl sulfate ($LiDodSO_4$) and polyacrylamide gel electrophoresis with this detergent at 4°C, two new chlorophyll–protein complexes were isolated. Under these conditions, the dissociation of pigment from the complexes is minimized. $LiDodSO_4$ was used because the sodium salt, previously used, precipitates in the cold. It is shown here that, in *R. sphaeroides* wild-type chromatophores, $LiDodSO_4$/polyacrylamide gel electrophoresis provides a convenient procedure for the isolation of the B875–antenna complex in addition to B850 and reaction centers that lack the heavy subunit.

MATERIALS AND METHODS

R. sphaeroides (NCIB 8253) was grown phototrophically at 30°C essentially as described (13) with 40-W General Electric incandescent lamps at a light intensity of 1800 lux as measured on a Weston model 756 illumination meter. Chromatophores were purified from French-press extracts as described (7). All membrane isolation procedures were carried out at 4°C; buffers contained 1.0 mM phenylmethylsulfonyl fluoride (PMSF) to inhibit protease activity.

Pigment–protein complexes were isolated by preparative gel electrophoresis on polyacrylamide slabs as described (12) with 0.1% $LiDodSO_4$ and 1.0 mM EDTA in the upper reservoir buffer. Samples contained chromatophores (1 mg of Bchl per ml), $LiDodSO_4$ in a 20:1 ratio (wt/wt) with Bchl, 50 mM dithiothreitol, 12% (wt/vol) sucrose, 62.5 mM Tris at pH 6.8, and PMSF and benzamidine each at 1.0 mM and 5 mM ϵ-aminocaproic acid as protease inhibitors. The stacking and separating (3 × 120 × 300 mm) gels consisted of 5 and 7.5% polyacrylamide, respectively; the ratio of polyacrylamide to *N,N'*-methylenebisacrylamide was 30:0.8. Some of the excised B875 antenna and reaction center complexes were purified further by a second electrophoresis in which the separating gel consisted of a gradient of 7.5–15% acrylamide stabilized by a 5–17.5% sucrose gradient. Complexes were eluted from gel slices by homogenization in 50 mM Tris/0.4 M glycine buffer containing the protease inhibitors. All operations were carried out at 4°C in the dark.

Procedures for pigment determinations have been described (7). Values obtained for spheroidene and spheroidenone were verified after separation by thin-layer chromatography (14). Protein was determined by the method of Bradford (15) in the presence of 0.1% $LiDodSO_4$ with bovine serum albumin as a standard. Absorption spectra were obtained with a Cary 14R

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: Bchl, bacteriochlorophyll *a*; B850 and B875, antenna complexes differentiated by absorption maxima near 850 and 875 nm, respectively; $Lau(NMe)_2O$, lauryl dimethylamine oxide; $DodSO_4$, dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride.

spectrophotometer. Fluorescence emission spectra were recorded with apparatus described previously (16). Fluorescence yields were based on the value of 0.1% for the 900-nm emission band of reaction centers at low redox potential (17).

$LiDodSO_4$ was obtained from Gallard, Schlesinger (Carle Place, NY). All other chemicals were reagent grade.

RESULTS

For elucidation of effects of $LiDodSO_4$ treatment at 4°C on *R. sphaeroides* chromatophores, detergent concentrations in the solubilization buffer were varied prior to electrophoresis at this temperature. It was found that a 1:20 (wt/wt) Bchl/$LiDodSO_4$ ratio solubilized the membrane efficiently while releasing only small amounts of free pigment; this ratio was used in all subsequent experiments. Lower Bchl/$LiDodSO_4$ ratios released more pigment; at higher ratios, the sample failed to enter the gel. Higher detergent concentrations also destroyed the labile B875 antenna complex.

Fig. 1 shows the pigmented bands observed after $LiDodSO_4$/polyacrylamide gel electrophoresis of chromatophores at 4°C. Up to 11 pigment-protein complexes were detected in unheated samples. Absorption spectra of three (designated B850, B875, and LM) were characteristic of single species of Bchl–protein complexes (Fig. 2). These illustrate the usefulness of this procedure for their simultaneous preparation and, most significantly, show that the B875 complex has now been isolated. The isolated complex absorbed maximally in the near-infrared, at 873 nm. The absence of a distinct absorption maximum near 800 nm indicates minimal contamination with reaction centers or B850. Absorption maxima at 500, 466, and 439 nm attributable to carotenoids are at shorter wavelengths relative to those in the isolated B850 complex and in chromatophores. After acetone/methanol extraction, no such difference was observed. The absorption spectrum of the B850 complex (Fig. 2 *Top*) is similar to that obtained for this component purified with lauryl dimethylamine oxide [$Lau(NMe)_2O$] and Triton (11). The spectrum of the LM gel band is comparable to that of reaction centers isolated after $Lau(NMe)_2O$ treatment of wild type (10). Chemical oxidation of the LM particles caused bleaching of the 860-nm band; however, light-induced absorbance measurements at 790 and 810 nm showed that only about 1% of these were photochemically active, perhaps because quinones that act as electron acceptors were reduced, deranged, or missing. The carotenoid absorption bands in the LM sample were also at shorter wavelengths than in B850.

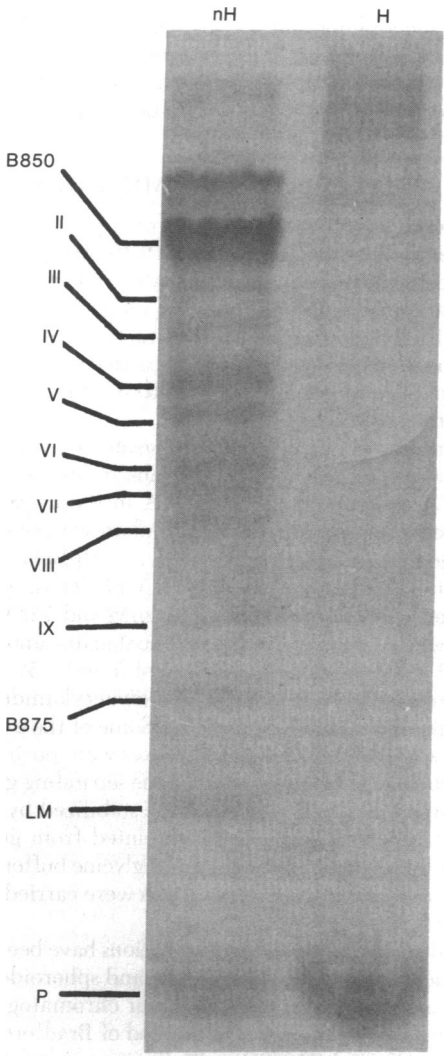

FIG. 1. Pigment-protein complexes from *R. sphaeroides* in an unstained gel. Chromatophores (20 μg of Bchl) were treated with $LiDodSO_4$ at 4°C for 60 sec; electrophoresis was on a 7.5% polyacrylamide gel at 4°C. II–IX, Complexes with various levels of B850 and B875 (see text); LM, reaction center particle; P, free pigment. nH, sample not heated; H, sample heated.

Near-infrared absorption spectra of the remaining pigmented bands (Fig. 3) showed that they contained various proportions of the B850 and B875 complexes, with the latter greatly enriched in complexes of higher electrophoretic mobility. Aside from LM, the B875 complex had the highest mobility in the gel.

The near-infrared fluorescence of the isolated B850 and B875 antennae was also examined (Fig. 4). Excitation of gel slices with 590-nm monochromatic light revealed major components that emitted maximally at 902 and 872 nm for the B875 and B850 complexes, respectively. These emission maxima agree with those resolved from the fluorescence spectrum of wild-type chromatophores (20) and with the maximum of isolated B850 (21, 22). The yields of fluorescence from B850 and B875 were found to be 23 and 17%, respectively, in agreement with those reported for an antenna complex isolated from *R. sphaeroides* (23). This may be fortuitous, however, because fluorescence yield varies strongly with detergent concentration (22). Such values represented an 8- to 10-fold increase over fluorescence yields of chromatophores (23).

The Bchl content of the isolated complexes is shown in Table 1. The B850 preparations showed the expected value of 3 Bchl/complex (23). The value of 2.4 Bchl per LM complex is decidedly less than the 4.0 expected (9), and the value of 1.6 Bchl/B875 (assuming a minimal complex of two polypeptides) falls short of the nearest integer, 2. Some Bchl may have been

Table 1. Bchl content of complexes isolated by $LiDodSO_4$/polyacrylamide gel electrophoresis*

	Bchl, μg/mg protein	Bchl/complex,† molar ratio
B850 complex	162.1	3.1 ± 0.4
B875 complex	74.6	1.64 ± 0.5
LM complex	72.4	2.42 ± 0.8
B850 complex‡	178	3.5
Chromatophores	73.3	—

* Values shown are means of several preparations.

† Calculated on the basis of two polypeptides for each complex. The molecular weights of the appropriate polypeptide components are in the text. Results are shown as mean ± SD.

‡ Prepared by the method of Clayton and Clayton (11).

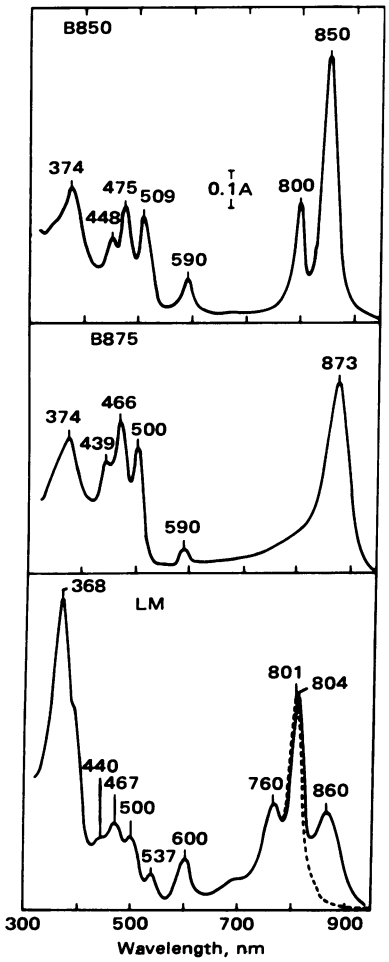

FIG. 2. Absorption spectra of the B850 and B875 antenna complexes and the reaction center complex (LM) isolated by LiDodSO$_4$/polyacrylamide gel electrophoresis. Spectra were obtained directly on the gel slices. For oxidation of the 860-nm reaction center band (dashed line), the gel slice was soaked in 10 mM potassium ferricyanide for 15 min.

lost during isolation and assay, and the protein estimations may have been in error because albumin is not necessarily a fair standard. With these reservations, we suggest that, in the minimal B875 complex, two molecules of Bchl are bound to two polypeptides. We found a molar ratio of 1.18 ± 0.09 carotenoid molecules per Bchl in the isolated B875 complex; this agrees with the value of 1.0 deduced by Sistrom (24) from the pigment composition of *R. sphaeroides* Ga membranes with various proportions of B850 and B875.

Low molecular weight polypeptides (M_r, 8000–14,000) have been observed by NaDodSO$_4$/polyacrylamide gel electrophoresis of antenna complexes from various species of Rhodospirillaceae (11, 25–28). The apoproteins of our B875 and B850 complexes were identified after eluted preparations were heated at 100°C for 60 sec in 1.0% LiDodSO$_4$. These polypeptide patterns were compared to those of unheated samples in LiDodSO$_4$/polyacrylamide gel electrophoresis at 4°C (Fig. 5 *Upper*). The unheated complexes remained largely intact as evidenced by comigration with their counterparts in unheated chromatophores. In contrast, heating resulted in complete dissociation of the isolated complexes into lower molecular weight polypeptides. This also was observed in chromatophores by intensification (after heating) of reaction center bands L and M and those near the gel front, concomitant with the disappearance of stained bands corresponding to pigmented com-

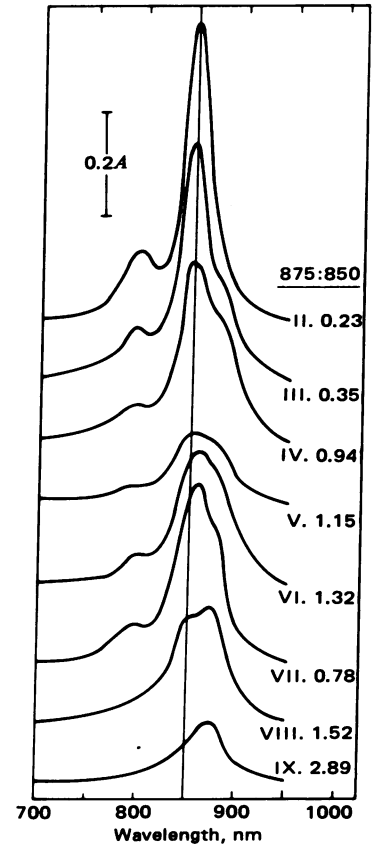

FIG. 3. Near-infrared absorption spectra of complexes II through IX. Spectra were corrected for light scattering by the gel slices. The B850 and B875 levels were calculated from the equations of Crounse *et al.* (18). The values obtained for the 875:850 ratio of the B850 and B875 complexes (Fig. 2) were 0.09 and 4.04, respectively.

plexes shown in Fig. 1. Electrophoresis at 4°C did not resolve low molecular weight components in heated chromatophores, presumably because of retarded migration of mixed pigment–phospholipid–detergent micelles at low temperature. Electrophoresis of heated samples at room temperature facilitated comparison of the low molecular weight polypeptides of the isolated complexes with those of chromatophores (Fig. 5 *Lower*); the latter were resolved into at least three components designated (29) LH-1 to LH-3 (apparent M_r, 12,000, 10,000, and 8000, respectively). Two polypeptides that migrated to the same positions as LH-1 and LH-3 were observed with the B875 complex whereas B850 complexes yielded components with electrophoretic mobilities similar to those of LH-2 and LH-3.

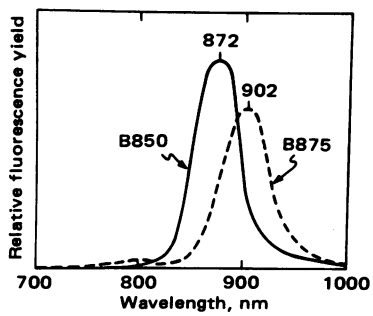

FIG. 4. Fluorescence emission spectra of the B850 and B875 pigment–protein complexes. Spectra were corrected by comparison with the known black-body emission spectrum of a tungsten filament at 2860 K (19). The small fluorescence emission near 800 nm in the B875 preparation is apparently due to free Bchl.

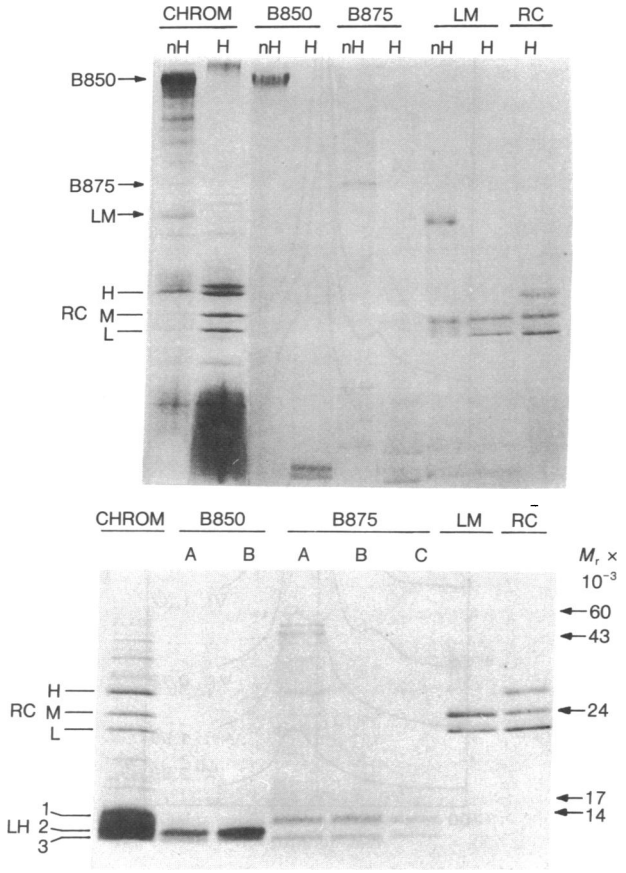

FIG. 5. Polypeptide composition of the Bchl–protein complexes. (*Upper*) Analysis by $LiDodSO_4$/polyacrylamide gel electrophoresis at 4°C on a 7.5–15% polyacrylamide gel (1.0 × 200 × 300 mm). CHROM, chromatophores (12 μg of Bchl); RC, *R. sphaeroides* R-26 reaction centers prepared as described (8); nH, samples not heated; H, heated at 100°C for 60 sec. Gels were stained with Coomassie brilliant blue as described (29). (*Lower*) Analysis of heated samples on a 10–14% polyacrylamide gel (1.5 × 100 × 150 mm) at room temperature. B850: A, prepared by $LiDodSO_4$/polyacrylamide gel electrophoresis; B, $Lau(NMe)_2O$/Triton treatment (11). B875: purified by one (A) or two (B, C) cycles of preparative electrophoresis with no protease inhibitors (A), with PMSF present (B), or with PMSF benzamidine, and ε-aminocaproic acid present (C). LM is shown after one cycle of preparative electrophoresis. H, L, and M are reaction center (RC) subunits [M_r, 28,000, 24,000, and 21,000 (9)]; LH-1 to LH-3, antenna–Bchl associated polypeptides (M_r, 12,000, 10,000, and 8000).

Fig. 5 *Lower* also shows that such polypeptide components are not artifacts of proteolytic digestion because no differences were observed when the protease inhibitors PMSF, benzamidine, and ε-aminocaproic acid were included in all buffers.

After heating, the complex with the spectral properties of reaction centers (designated LM) was found to contain only the M_r 24,000 (M) and 21,000 (L) polypeptide subunits (9). The absence of the H subunit (apparent M_r, 28,000) is an agreement with earlier observations (9, 14, 30) that denaturation of reaction centers with $NaDodSO_4$ under mild conditions gives rise to LM particles, the smallest photochemically active unit in *R. sphaeroides* (30,31).

DISCUSSION

$LiDodSO_4$/polyacrylamide gel electrophoresis at 4°C provides a convenient and rapid procedure for detection and isolation of pigment–protein complexes present in the chromatophore membrane of *R. sphaeroides*. Three of these complexes have been identified as B850 and B875 antenna components and a reaction center preparation. Previously, the B875 pigment–protein complex from the wild type had been observed in absorption spectra overlapping with varying levels of B850 (6), and the then available membrane fractionation methods had resulted in its denaturation (11, 23). At least eight additional pigmented complexes were observed, and spectral properties indicate that they were composed of varying proportions of B850 and B875. It is possible that the fractions isolated by this relatively mild electrophoretic procedure (cf. ref. 12) represent native Bchl–protein aggregates and as such reflect the *in vivo* organization of the photosynthetic apparatus.

The compositions and sizes of the isolated complexes are consistent with a model proposed by Monger and Parson (32) for their arrangement within the membrane, based on fluorescence properties. In accord with a pathway of energy transfer from B850 through B875 to reaction centers (20, 33), it was proposed that B875 interconnects and surrounds reaction centers and that the vast array of B850 is arranged peripherally in larger "lakes" (32). A close association between the reaction centers and B875 has been suggested by their fixed stoichiometry in cells of varying pigment contents (6) and the observation that these are the first complexes synthesized during repigmentation (7). Our finding that larger complexes are enriched in B850 whereas smaller ones are enriched in B875 is compatible with this model. Complexes with varying proportions of the two antenna components may be derived from membrane areas in which they are intermingled. The apparent sizes in which various complexes are isolated may reflect the manner in which photosynthetic units are expanded by addition (6) of an outer array (32) of B850 complexes.

In accord with the polypeptide composition reported for other antenna preparations (11, 25, 28), three polypeptide bands observed near the gel front in purified chromatophore preparations were designated (29) as LH-1 to LH-3 (apparent M_r, 12,000, 10,000, and 8000, respectively). LH-1 and LH-2 were identified here as components of B875 and B850, respectively, and a polypeptide with electrophoretic mobility essentially identical to that of LH-3 was found in both complexes. It is not yet clear whether the latter polypeptide represents the same component in both of the complexes or different components migrating to the same position in gels. This is in contrast to antenna complexes isolated from *R. capsulata* mutants (28) in which B850 contained a M_r 14,000 polypeptide component, in addition to apparent M_r 8000 and 10,000 components and a single M_r 12,000 polypeptide was observed with a "B875" complex. Recent proteolytic digestion studies suggested that Bchl in association with M_r 10,000 and 8000 polypeptides is responsible for the absorption maxima near 850 and 800 nm, respectively (34). Because the B875 complex isolated here contains no B850 and no M_r 10,000 polypeptide, it is unlikely that the apparent M_r 8000 polypeptide in this preparation arose from B850 contamination. An immunodiffusion analysis with anti-chromatophore antiserum shows that the purified B850 and B875 complexes share a common precipitin line but it is not yet known whether this represents the LH-3 polypeptide (unpublished results).

With regard to the pigment composition of the antenna complexes isolated here, three molecules of Bchl were found associated with the B850 preparation, whereas with B875, approximately two Bchls were detected, subject to the reservations expressed earlier. The composition observed for B850 agrees with that reported for other *R. sphaeroides* preparations (23, 25); spectral observations indicated that the 850-nm absorption band contains two exciton-coupled Bchl molecules whereas a single Bchl molecule is associated with the 800-nm band (35). The finding that nearly two molecules of Bchl are associated

with the B875 complex agrees with the value reported for the antenna complex isolated from the carotenoidless *R. sphaeroides* mutant R-26 (23). It is possible that the antenna observed in this mutant represents a mutated B875 because no B800 band is found (24). Furthermore, the ratio of antenna Bchl to reaction centers in R-26 is about 30:1, independent of growth conditions, as observed for B875 in the wild type (6).

Our findings suggest that the pigment composition of the isolated B875 complex largely reflects that of B875 within the membrane. It had been predicted (24) that, in the B875 complex, carotenoids should exist with Bchl in a fixed molar ratio near 1.0; this was observed here. Furthermore, the positions of the near-infrared absorption and fluorescence emission maxima are close to those deduced from measurements made on membrane preparations with various B850 and B875 levels (5, 20). Finally, the position of carotenoid absorption maxima in our B875 preparation at shorter wavelengths than the corresponding maxima in B850 agrees with spectra of certain mutants of *R. capsulata* isolated by B. L. Marrs (personal communication). In mutant strain MW442, which contains B875 but lacks B850, the carotenoid absorption maxima are at shorter wavelengths (by 7–8 nm) than in strains in which B850 is predominant.

It is anticipated that the mild solubilization technique outlined here will be applicable to other photosynthetic bacteria and that hitherto incompletely resolved "B890" complexes (36) can now be examined in more detail. In addition, the complexes II–IX which contain the B875 and B850 antenna components still associated may provide useful experimental models for the study of energy migration between different antenna components.

We thank Barry Marrs for allowing us to cite his results prior to publication. We are grateful to Michael Kendall-Tobias for providing the R-26 reaction centers and to Larry Martin for skilled technical assistance. This study was supported by National Science Foundation Grants PCM76-24142 (R.A.N.) and PCM78-11102 (R.K.C.), U.S. Public Health Service Grants GM26248 (R.A.N.) and GM21060 (N.-H.C.) from the National Institute of General Medical Sciences and Contract EY-76-S-02-3162 with the U.S. Department of Energy (R.K.C.). Fellowships were provided to R.M.B. and C.N.H. from the Charles and Johanna Busch Memorial Fund Award and to P.D. by Ecole Normale Supérieure, Paris. R.A.N. and N.-H.C. were the recipients of U.S. Public Health Service Research Career Development Awards GM00093 and GM00223, respectively.

1. Cohen-Bazire, G. & Sistrom, W. R. (1966) in *The Chlorophylls*, eds. Vernon, L. P. & Seeley, G. R. (Academic, New York), pp. 313–341.
2. Niederman, R. A. & Gibson, K. D. (1978) in *The Photosynthetic Bacteria*, eds. Clayton, R. K. & Sistrom, W. R. (Plenum, New York), pp. 78–118.
3. Clayton, R. K. (1971) *Adv. Chem. Phys.* **19,** 353–378.
4. Blankenship, R. E. & Parson, W. W. (1978) *Annu. Rev. Biochem.* **47,** 635–653.
5. Sistrom, W. R. (1964) *Biochim. Biophys. Acta* **283,** 492–504.
6. Aagaard, J. & Sistrom, W. R. (1972) *Photochem. Photobiol.* **15,** 209–225.
7. Niederman, R. A., Mallon, D. E. & Langan, J. J. (1976) *Biochim. Biophys. Acta* **440,** 429–447.
8. Clayton, R. K. & Wang, R. T. (1971) in *Methods Enzymol.* **23,** 696–704.
9. Okamura, M. Y., Steiner, L. A. & Feher, G. (1974) *Biochemistry* **13,** 1394–1403.
10. Jolchine, G. & Reiss-Husson, F. (1974) *FEBS Lett.* **40,** 5–8.
11. Clayton, R. K. & Clayton, B. J. (1972) *Biochim. Biophys. Acta* **283,** 492–504.
12. Delepelaire, P. & Chua, N.-H. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 111–115.
13. Parks, L. C. & Niederman, R. A. (1978) *Biochim. Biophys. Acta* **511,** 70–82.
14. Cogdell, R. J., Parson, W. W. & Kerr, M. A. (1976) *Biochim. Biophys. Acta* **430,** 83–93.
15. Bradford, M. (1976) *Anal. Biochem.* **72,** 248–254.
16. Wraight, C. A. & Clayton, R. K. (1972) *Biochim. Biophys. Acta* **333,** 246–260.
17. Zankel, K. L., Reed, D. W. & Clayton, R. K. (1968) *Proc. Natl. Acad. Sci. USA* **61,** 1243–1249.
18. Crounse, J., Sistrom, W. R. & Nemser, S. (1963) *Photochem. Photobiol.* **2,** 361–375.
19. Clayton, R. K. (1970) in *Light and Living Matter* (McGraw-Hill, New York), Vol. 1, pp. 122–125.
20. Zankel, K. L. & Clayton, R. K. (1969) *Photochem. Photobiol.* **9,** 7–15.
21. Hunter, C. N., van Grondelle, R., Holmes, N. G., Jones, O. T. G. & Niederman, R. A. (1979) *Photochem. Photobiol.* **30,** 313–316.
22. Heathcote, P. & Clayton, R. K. (1977) *Biochim. Biophys. Acta* **549,** 506–515.
23. Sauer, K. & Austin, L. A. (1978) *Biochemistry* **17,** 2011–2019.
24. Sistrom, W. R. (1978) in *The Photosynthetic Bacteria*, eds. Clayton, R. K. & Sistrom, W. R. (Plenum, New York), pp. 841–848.
25. Fraker, P. J. & Kaplan, S. (1972) *J. Biol. Chem.* **247,** 2732–2737.
26. Firsow, N. N. & Drews, G. (1977) *Arch. Microbiol.* **115,** 299–306.
27. Cuendet, P. A. & Zuber, H. (1977) *FEBS Lett.* **79,** 96–100.
28. Feick, R. & Drews, G. (1978) *Biochim. Biophys. Acta* **501,** 499–513.
29. Broglie, R. M. & Niederman, R. A. (1979) *J. Bacteriol.* **138,** 788–798.
30. Feher, G. (1971) *Photochem. Photobiol.* **14,** 373–387.
31. Feher, G. & Okamura, M. Y. (1978) in *The Photosynthetic Bacteria*, eds. Clayton, R. K. & Sistrom, W. R. (Plenum, New York), pp. 349–386.
32. Monger, T. G. & Parson, W. W. (1977) *Biochim. Biophys. Acta* **460,** 393–407.
33. Vredenberg, W. J. & Duysens, L. N. M. (1963) *Nature (London)* **197,** 355–357.
34. Feick, R. & Drews, G. (1979) *Z. Naturforsch.* **34,** 196–199.
35. Cogdell, R. & Crofts, A. R. (1978) *Biochim. Biophys. Acta* **502,** 409–416.
36. Thornber, J. P., Trosper, T. L. & Strouse, C. E. (1978) in *The Photosynthetic Bacteria*, eds. Clayton, R. K. & Sistrom, W. R. (Plenum, New York), pp. 133–160.