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PARTIAL PURIFICATION, SUBUNIT STRUCTURE AND THERMAL STABILITY OF THE PHOTOCHEMICAL REACTION CENTER OF THE THERMOPHILIC GREEN BACTERIUM *CHLOROFLEXUS AURANTIACUS*

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Spectrally pure reaction center preparations from *Chloroflexus aurantiacus* have been obtained in a stable form; however, the product contained several contaminating polypeptides. The reaction center pigment molecules (probably three bacteriochlorophyll *a* and three bacteriopheophytin *a* molecules) are associated with two polypeptides (M_r = 30000 and 28000) in a reaction center complex of M_r = 52000. No carotenoid is present in the complex. These data together with previous spectral data suggest that the *Chloroflexus* reaction center represents a more primitive evolutionary form of the purple bacterial reaction center, and that it has little if any relationship to the green bacterial component. A reaction center preparation from *Rhodopseudomonas sphaeroides* R26 was fully denatured at 50°C while the *Chloroflexus* reaction center required higher temperatures (70–75°C) for complete denaturation. Thus, an intrinsic membrane protein of a photosynthetic thermophile has been demonstrated to have greater thermal stability than the equivalent component of a mesophile.

Chloroflexus aurantiacus is a filamentous gliding photosynthetic bacterium that may be closely related to the common ancestor of all other photosynthetic bacteria [1,2]. This organism is unique among the known photosynthetic bacteria in being a thermophile with an optimum temperature for growth near 60°C. The photosynthetic apparatus of *Chloroflexus* has a unique combination of components similar to those found separately in the Rhodospirillaceae and the Chlorobiaceae [1].

In *Chloroflexus,* antenna BChl *c* absorbing at 740 nm (BChl *c*-740) funnels excitation energy to the photochemical reaction center (P-865) via BChl *a* complexes absorbing at 790, 800 and 865 nm [3]. BChl *c*-740 and BChl *a*-790 plus several carotenoids are contained in chlorosomes, which appear similar in structure and function to those in green sulfur bacteria [4–7]. BChl *a*-800, BChl *a*-865, the photochemical reaction center complex and associated electron-transport components, including cytochrome *c*-553, are located in this cell membrane [5,7]. These membrane-bound components are spectrally very similar to the equivalent compo-

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Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin.

Supplementary data to this article are deposited with, and can be obtained from, Elsevier Biomedical Press B.V., BBA Data Deposition, P.O. Box 1345, 1000 BH Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/252/40021/723 (1983) 322. The supplementary information includes results using eleven different surfactants for purification of antenna complexes and the reaction center; rationale and experiences leading to adoption of final purification procedure for reaction center [8].

nents found in the intracellular membranes in purple bacteria (cf. Ref. 8).

We have recently reported the isolation of the photochemical reaction center of *Chloroflexus* and have studied its spectral characteristics [8]. While such characteristics are particularly reminiscent of purple bacterial reaction centers, there were some distinct differences from them, e.g., the molar ratio of BChl *a* to BPh is one while it is two in the purple bacterial reaction center. We continue the comparison between *Chloroflexus* and *Rhodopseudomonas sphaeroides* reaction centers here by examining some of their biochemical characteristics; in particular, their subunit structure and thermal stability are compared. The latter was studied because of the availability, for the first time, of photochemical reaction centers from a thermophile to compare with those of a mesophile. Few intrinsic membrane proteins from thermophiles have been studied [9].

Growth of cells and preparation of crude extracts: *C. aurantiacus* strain J-10-fl (ATCC 29366) was grown, harvested, and stored as previously described [8]. Crude cell extracts were prepared by sonication of cells in Tris-HCl buffer (50 mM), pH 8.0 or 9.0 [8].

Preparation of reaction centers: Reaction centers were prepared from crude extracts at pH 9.0 by extraction with lauryldimethylamine *N*-oxide (1%, w/v) and chromatography on DEAE-cellulose (Whatman DE53). Full details are given in Refs. 8 and 11.

SDS-polyacrylamide gel electrophoresis: SDS-polyacrylamide gel electrophoresis was done according to the method of Laemmli [10] with a 12.5% acrylamide running gel. All samples were incubated in a denaturing buffer (Tris-HCl) containing 3% (w/v) SDS and 5% (v/v) mercaptoethanol. Reaction centers were incubated at 20 or 100°C for 2 min prior to loading on the gel. On some occasions colored reaction center-protein bands were cut out after electrophoresis but before staining with Coomassie blue (0.1%)/acetic acid (10%)/isopropanol (30%). These excised bands were boiled for 3 min in buffer containing 3% SDS and 0.1 M dithiothreitol before loading on a 10% acrylamide gel containing 8 M urea. Gels were destained in acetic acid (7%) and methanol (5%). Marker proteins were bovine serum albumin, catalase, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme.

Thermal stability: The effects of temperature were assessed by incubating reaction centers (A_{865} = 0.03) in 50 mM Tris-HCl buffer (pH 8.0 at room temperature) containing no more than 0.05% (w/v) lauryldimethylanine *N*-oxide at a given temperature for 15 min, immediately cooling on ice and then recording the absorption spectrum at 20°C. Reversible oxidation of P-865 in the incubated solution was measured using $K_3Fe(CN)_6$ as oxidant and sodium ascorbate as reductant.

Spectroscopy: Absorption spectra were recorded with an Aminco DW-2 spectrophotometer. A band pass of 3.0 nm was used.

Isolation of the photochemical reaction center: Data on our experiences with the use of 11 different surfactants for the purification of antenna complexes and the reaction center have been deposited in the BBA Data Bank. Also deposited are details on the rationale and experiences which led to the final purification procedure for the reaction center [8] being adopted.

Subunit structure of the reaction center: Fig. 1 shows the polypeptide bands obtained after electrophoresis of dissociated, partially purified (twice chromatographed and dialyzed) reaction centers of *C. aurantiacus* strain J-10-fl (lanes 4 and 5) and

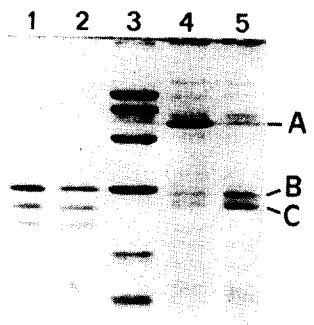

Fig. 1. Polypeptides seen after SDS-polyacrylamide gel electrophoresis in a 12.5% gel. Lane 1, purified reaction centers from *Rps. sphaeroides* R-26, unheated. Lane 2, purified reaction centers from *Rps. sphaeroides* R-26, boiled for 2 min. Lane 3, protein standards (see text). Lane 4, reaction center preparation from *C. aurantiacus* J-10-fl, unheated. Lane 5, reaction center preparation from *C. aurantiacus* J-10-fl, boiled for 2 min. (A) M_r = 52000; (B) M_r = 30000, (C) M_r = 28000.

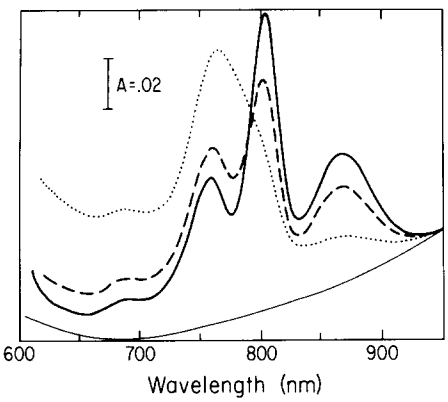

Fig. 2. Near-infrared absorption spectra (with baseline) of reaction centers purified from *Rps. sphaeroides* R-26. Aliquots of the preparation were incubated in buffer at various temperatures for 15 min, cooled and spectra recorded at 20°C. (———) 20, (- - - - - -) 45, (· · · · · ·) 50°C.

those from purified reaction centers of *Rps. sphaeroides* R-26 (lanes 1 and 2). Reaction centers from *Chloroflexus* electrophoresed differently depending on whether or not they were heated at 100°C in the denaturing buffer. Without such heating and prior to staining the gel, the reaction center pigments are located in a single, prominent gray band with an apparent M_r of 52000 (lane 4). The spectrum of this band was identical to that of the isolated reaction center (data not shown). When the reaction center preparation is boiled (lane 5), the M_r 52000 band was eliminated and two intensely staining bands with apparent M_r of 30000 and 28000 now appeared. Heating reaction centers at 45 and 55°C in the same buffer for 2 min was not sufficient to dissociate the M_r 52000 complex (see below). Although the band at M_r 52000 was the most heavily stained in all gels of unboiled reaction centers, traces of the M_r 30000 and 28000 bands (apoproteins of the reaction center?) were also present. The reaction center-protein complex was not electrophoretically pure and other weakly staining bands (three of larger molecular weight and about six of lower molecular weight) were present. Further, some material was left at the top of the gel whether or not the samples were boiled. When the M_r 52000 dalton band was excised, dissociated (see above) and then loaded on a 10% SDS-acrylamide gel containing 8 M urea, three bands were present with apparent M_r of 52000–54000, 30000, and 27000—28000. No other bands were present, and no material was left at the top of the gel.

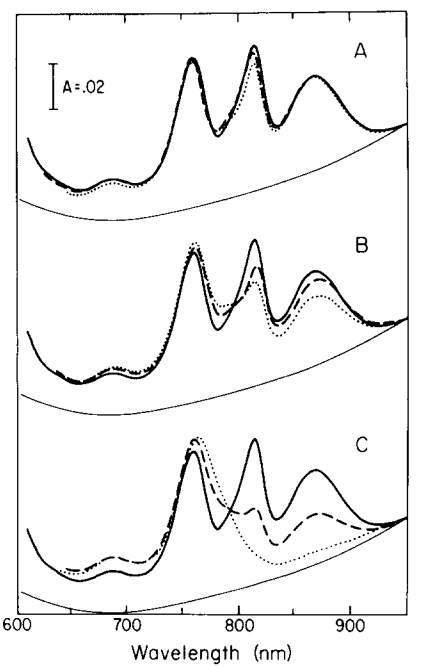

Fig. 3. Near-infrared absorption spectra (with baselines) of reaction centers isolated from *C. aurantiacus* J-10-fl. Reaction centers were incubated in buffer at various temperatures for 15 min, cooled, and spectra recorded at 20°C. A: (———) 20, (- - - - - -) 50, (· · · · · ·) 55°C; B: (———) 20, (- - - - - -) 60, (· · · · · ·) 65°C; C: (———) 20, (- - - - - -) 70, (· · · · · ·) 75°C.

Further purification of the reaction centers was attempted by ammonium sulfate fractionation. Some reaction center was precipitated between 10 and 20% saturation, and most (60% of the initial A_{865}) was precipitated at 35% saturation. It was necessary to include a minimum of 0.05% lauryldimethylamine *N*-oxide in the buffer to redissolve the precipitated reaction centers which were dark gray. Electrophoresis on 12.5% acrylamide gels revealed that most extraneous peptides were still present in the preparation.

Thermal stability: Fig. 2 shows the absorption spectra at 20°C of three identical samples of reaction centers from *Rps. sphaeroides* R-26. The control was kept at 20°C for 15 min. The others were heated to 45 and 50°C for 15 min, then immediately cooled to 20°C and the spectra recorded. Heating at 45°C resulted in decreases in A_{865} and A_{800} and an increase in A_{760}. Heating at

50°C, even for only 10 min (data not shown), resulted in precipitation of the reaction center and dissociation of the pigments from the protein complex. Fig. 3 shows the same experiment performed with reaction centers of *C. aurantiacus* J-10-fl. There is a slight decrease in A_{815} after heating at 50 and 55°C but there is no effect on A_{757} or A_{865}. The spectrum resulting from 45°C incubation is not shown because it was identical to the control at 20°C. A progressive decrease occurs in the height of the 815 and 865 nm bands and A_{757} and $A_{770-780}$ increase in samples heated at 60 and 65°C (Fig. 3). There is a further intensification of these changes at 70°C and complete dissociation at 75°C. Similarly, the amount of P-865 decreases when heated at 60 and 70°C and none remains when heated at 75°C (data not shown).

The studies described here and our previous work [8] show that the photochemical reaction center of *Chloroflexus* is much more closely related to the purple than the green bacterial reaction center. The *Chloroflexus* reaction center shows great dissimilarities in absorbance and subunit composition from reaction center complexes obtained from other green bacteria [4].

However, interesting differences even exist between this component in *Chloroflexus* and that in purple bacteria: (a) The *Chloroflexus* reaction center is composed of three BChl *a* and three BPh *a* molecules [8] associated with two polypeptides of 30 and 28 kDa (Fig. 1); (b) No carotenoid is present in the most purified material, the 52 kDa band (Fig. 1); (c) Denaturation of the pigment protein-reaction center complex and its P-865 requires temperatures above 55°C, and complete denaturation only occurs after heating to 75°C (Fig. 3); (d) The reaction center is not dissociated by the standard electrophoretic procedure of Laemmli [10] (Fig. 1). On the other hand, reaction centers from BChl *a*-containing purple bacteria have four BChl and two BPh molecules associated with three polypeptides (L, M and H) in the 20–30 kDa range (Fig. 1) [12], and complete denaturation of the complex occurs at 50°C and in the procedure of Laemmli [10], as expected for proteins of mesophilic organisms.

From the data available here and in Ref. 8 we conclude that the *Chloroflexus* reaction center may be an earlier version of the purple bacterial reaction center. The significance of one BChl *a* molecule apparently substituting for a BPh molecule and the addition of a third polypeptide to the reaction center complex during the evolution of purple bacterial reaction centers is unknown. It is tempting to equate the 30 and 28 kDa polypeptides of the *Chloroflexus* reaction center with the M and L subunits [13] of the purple bacterial component, since all the chromophore molecules in the latter have been shown to be associated only with two (M and L) polypeptides [12,13]. Immunochemical and/or sequence studies will reveal their correct phylogenetic relationship. The difference in thermal stability between the two reaction center preparations (Figs. 2 and 3) confirms expectations that hydrophobic intrinsic membrane proteins, as well as soluble proteins, have increased thermal stability in thermophilic organisms [9]. Recent studies on some of the photochemical reactions in membranes of *C. aurantiacus* [14] are also consistent with the notion that this reaction center has several features in common with those of the purple bacteria.

The stability of the reaction center complex during electrophoresis proved fortunate. It revealed that all the spectral characteristics previously described for the *Chloroflexus* reaction center [8] are associated with a single complex with an apparent size of 52 kDa and having two apoproteins of 30 and 28 kDa probably present in a 1 : 1 ratio, and furthermore that the *Chloroflexus* reaction center was stable when its protein was represented by only two polypeptides (cf. LM stability in *Rps. sphaeroides* reaction centers). Electrophoresis also showed that the purification procedure used does not yield pure reaction centers, since at least nine contaminating polypeptides are present (Fig. 1). Ammonium sulfate precipitation improved the purity somewhat, but further work is required to obtain large amounts of this reaction center in pure form.

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