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On the role of the light-harvesting B880 in the correct insertion of the reaction center of *Rhodobacter capsulatus* and *Rhodobacter sphaeroides*

W. Jim Jackson, Patricia J. Kiley[+], Copper E. Haith, Samuel Kaplan[+] and Roger C. Prince

EXXON Research and Engineering Co., Annandale, NJ 08801 and [+] *Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

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The purple, non-sulfur photosynthetic bacteria *Rhodobacter capsulatus* and *Rhodobacter sphaeroides* have two types of pigment-protein complexes that absorb incident light and funnel it to the photochemical reaction center. One of these, B880, is present at an essentially constant ratio to the reaction center, while the abundance of the other, B800–850, varies with growth conditions. Independent work in our two laboratories has indicated that while the absence of B800–850 permits photosynthetic growth in both organisms, the lack of B880 produces a different phenotype in the two species. Thus *R. sphaeroides* is still photosynthetically competent when it lacks this complex, while *R. capsulatus* is not. This unanticipated difference in what appear to be very closely related organisms has caused us to reexamine the properties of the two mutants simultaneously, under identical conditions. We find that the original descriptions are indeed correct; the reaction center of *R. capsulatus* is not correctly inserted into the photosynthetic membrane in the absence of B880, while that of *R. sphaeroides* is.

Reaction center insertion; Pigment-protein complex; (*Rhodobacter*)

1. INTRODUCTION

Purple, non-sulfur photosynthetic bacteria have a variety of antenna pigment-protein complexes that absorb incident light and funnel it to the photochemical reaction center. *Rhodobacter capsulatus* and *Rhodobacter sphaeroides* are a closely related pair of organisms that have two of these pigment-protein complexes; B880, which has maximal absorbance at approximately 880 nm, and B800-850, which has two absorption maxima near 800 and 850 nm. B880 seems to be produced with a fixed stoichiometry to the reaction center of about 20:1, while B800-850 varies inversely with light intensity. Both complexes consist of two homologous but distinct peptides, the α- and β-subunits, in equimolar amounts, and bind 2–3 molecules of bacteriochlorophyll and 1–2 molecules of carotenoid per α-β pair. Excellent reviews of this subject are provided in [1,2].

Mutants lacking either or both of the antenna complexes are well known. The absence of B800-850 does not prevent photosynthetic growth, but the absence of B880 seems to have different effects in the two organisms. Meinhardt et al. [3] found that a B880-deficient strain of *R. sphaeroides* was photosynthetically competent, albeit at a somewhat lower quantum efficiency than the wild type, while Jackson et al. [4] found that a B880-deficient strain of *R. capsulatus* was unable to grow photosynthetically. Since the two organisms are often found to have very similar photosynthetic systems, these disparate results were unexpected. One possible explanation was that the *R. sphaeroides* mutant might still contain low levels of the B880 complex, perhaps the 15%

Correspondence address: W.J. Jackson, EXXON Research and Engineering Co., Annandale, NJ 08801, USA

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identified by Kramer et al. [5] as being closest to the reaction center. Meinhardt et al. [3] did not present low temperature spectra of their mutant, nor the results of polyacrylamide gel electrophoresis, and it was possible that the spectrum of the B800–850 complex might obscure low levels of B880 in room temperature spectra. We have now grown the two B880-deficient strains and compared their properties, including low temperature spectra and polyacrylamide gel analysis of their polypeptide profiles. We find that the two organisms do indeed show the dichotomous response to the absence of the B880 pigment-protein complex described in the original papers.

2. MATERIALS AND METHODS

R. sphaeroides RS2 ($B880^+$) and RS103 ($B880^-$) [3] and *R. capsulatus* PJS108 ($B880^+$) and PBS108 ($B880^-$) [4] were grown aerobically in a shaking incubator on the media described [3,4]. Chromatophores were prepared with a French pressure cell. Optical spectra were taken with a Hitachi 557 spectrometer equipped with a liquid nitrogen cryostat, and flash-induced spectral changes were measured with the rapidly responding spectrometer described elsewhere [6]. SDS-polyacrylamide gel electrophoresis was performed as described [4].

3. RESULTS AND DISCUSSION

The optical absorption spectra, measured near 77 K, of the four strains used in this work are shown in fig.1. The *R. sphaeroides* strains possess wild-type carotenoids, while the *R. capsulatus* strains are both 'green mutants' which are unable to synthesize wild-type carotenoids due to a mutation in the *crt*D gene [7]. Both wild-type strains, RS2 and PJS108, clearly possess the B880 spectral feature, which shifts to 890 nm at low temperatures, while both mutants, RS103 and PBS108, clearly lack it. As expected, all strains contained normal levels of the B800–850 complex.

Analysis of the protein subunits by SDS-gel electrophoresis shows that both RS103 and PBS108 lack the 12 kDa α-subunit of B880 (fig.2). Even though the β-subunits of B800–850 and B880 are not fully resolved in the controls, it is clear that both mutants are also missing the 6 kDa β-subunit of B880.

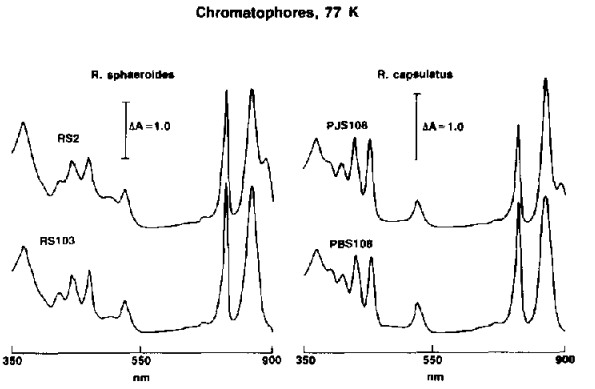

Fig.1. Absorption spectra of the four strains used in this work, measured near 77 K [4]. Chromatophores (approx. 200 μM bacteriochlorophyll) in 20 mM *N*-morpholino propanesulfonate, 100 mM KCl, pH 7.0, were mixed with an equal volume of glycerol, transferred to a mylar cuvette (1 mm path length), and frozen at 77 K.

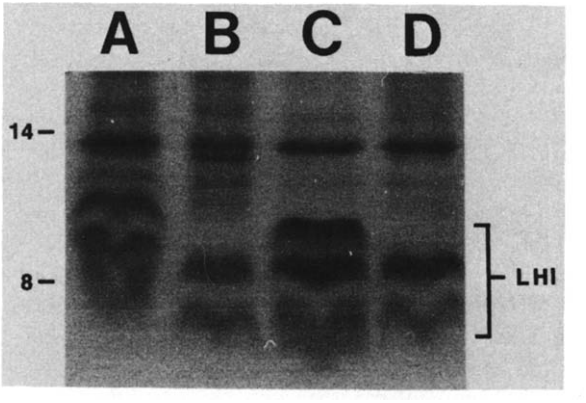

Fig.2. Coomassie blue-stained SDS-polyacrylamide gradient gel (10–20%) of chromatophores. Only the lower third of the gel, containing the antenna subunits, is shown. Lanes: A, *R. sphaeroides* RS2; B, *R. sphaeroides* RS103; C, *R. capsulatus* PJS108; D, *R. capsulatus* PBS108. LHI designates the B880 α- and β-subunits. Numbers to the left denote the mobility of molecular mass markers.

We thus conclude that both *R. sphaeroides* RS103 and *R. capsulatus* PBS108 lack the B800 antenna complex, as originally described [3,4]. We also confirm that of the four strains used in this work, only *R. capsulatus* PBS108 is photosyn-

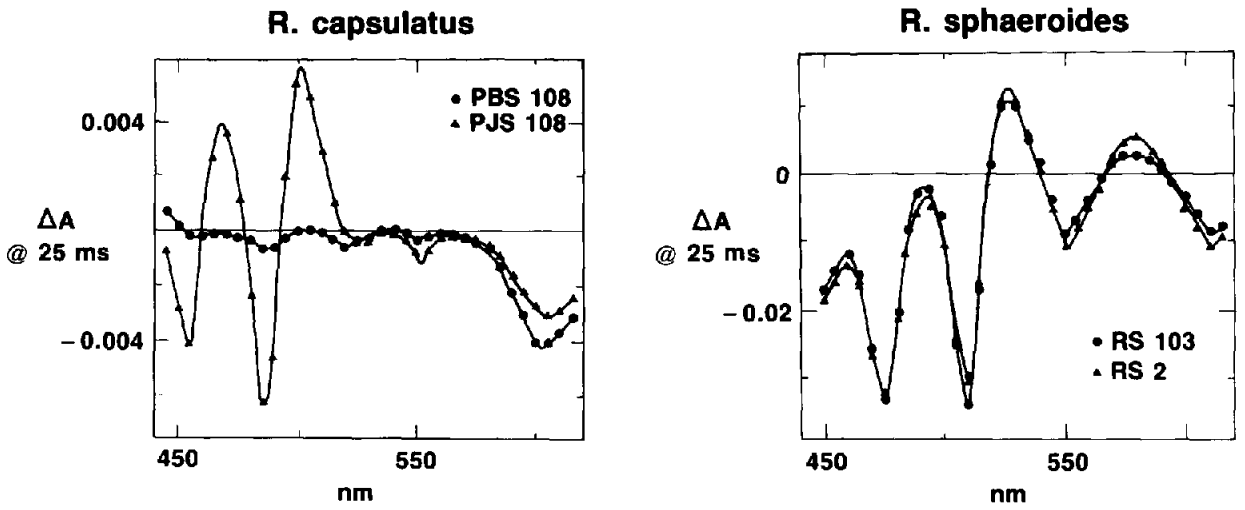

Fig.3. Flash-induced absorbance changes in the four strains used in this work. Chromatophores (19 μM BChl for *R. capsulatus*; 23 μM for *R. sphaeroides*) were suspended in 20 mM *N*-morpholinopropane sulfonate, 100 mM KCl, pH 7.0, with a few grains of solid sodium ascorbate to bring the E_h to about 150 mV. The spectra were measured in double-beam mode at the indicated wavelength minus 540 nm, 25 ms after a saturating single-turnover flash [6].

thetically incompetent. Fig.3 shows why this is so. All four strains possess fully photoactive photochemical reaction centers, as shown by the flash-induced decrease in absorbance at 605 nm. Both strains of *R. sphaeroides* display the well known light-induced carotenoid bandshift [8], as does *R. capsulatus* PJS108. The B880-strain of *R. capsulatus*, PBS108, does not. The carotenoid bandshift is an electrochromic response of the carotenoid pigments of the B800–850 complex [7] to transmembrane electrical potentials [8], and its presence in the three photosynthetically competent strains is a monitor of the membrane potential generated by cyclic photosynthetic electron transfer reactions within both the reaction center and the cytochrome bc_1 complex [8]. Since the carotenoids of the B800–850 complex of *R. capsulatus* PBS108 are capable of responding to transmembrane potentials induced by pulses of valinomycin and potassium, the absence of the shift indicates that the reaction centers in this strain are not doing useful electrical work [4]. Indeed, as we discussed in our earlier paper, the reaction centers appear to be oriented along the membrane rather than across it [4].

In conclusion, we find that both *R. capsulatus* PBS108 and *R. sphaeroides* RS103 do indeed lack the B880 complex, but that this defect gives rise to a photosynthetically incompetent phenotype only in *R. capsulatus*. This is because the reaction center of *R. capsulatus* is not correctly inserted into the membrane in the absence of B880, while this is not the case in *R. sphaeroides*. A dichotomy of phenotype in the two organisms is also seen with cytochrome c_2; the *R. capsulatus* strain lacking the cytochrome is photosynthetically competent [6], while the *R. sphaeroides* equivalent strain is photosynthetically incompetent [9]. This reminds us that while the two species share much in common in terms of their photosynthesis, it is unwise to generalize too broadly when predicting phenotypes from genotypes.

ACKNOWLEDGEMENT

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