ISOLATION OF A REACTION CENTER FRACTION FROM RHODOPSEUDOMONAS SPHEROIDES

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Sensitive spectrophotometric techniques have been used to detect light-induced absorbance changes in chromatophores of purple photosynthetic bacteria. These changes have been attributed to oxidation of the reaction center bacteriochlorophylls, P870 or P890 (Duysens, 1952; Goedheer, 1960; Clayton, 1962a), oxidation of cytochromes (Duysens, 1954; Smith and Ramirez, 1959; Clayton, 1962b; Olson, 1962), and reduction of ubiquinone (Clayton, 1962c). Treatment of chromatophores with K_2IrCl_6 isolates the reaction center components, P800 and P870, as spectral entities by destroying the light-harvesting bacteriochlorophyll (Clayton and Sistrom, 1966; Kuntz et al., 1964). However, this procedure does not purify the reaction center chlorophylls with respect to other chromatophore constituents. In this paper we will describe a method which has been successfully utilized to purify the reaction center components with respect to both spectral and chemical composition. We describe a preparation from Rhodopseudomonas spheroides, strain R-26, but the method has also been applied successfully to other purple bacteria.

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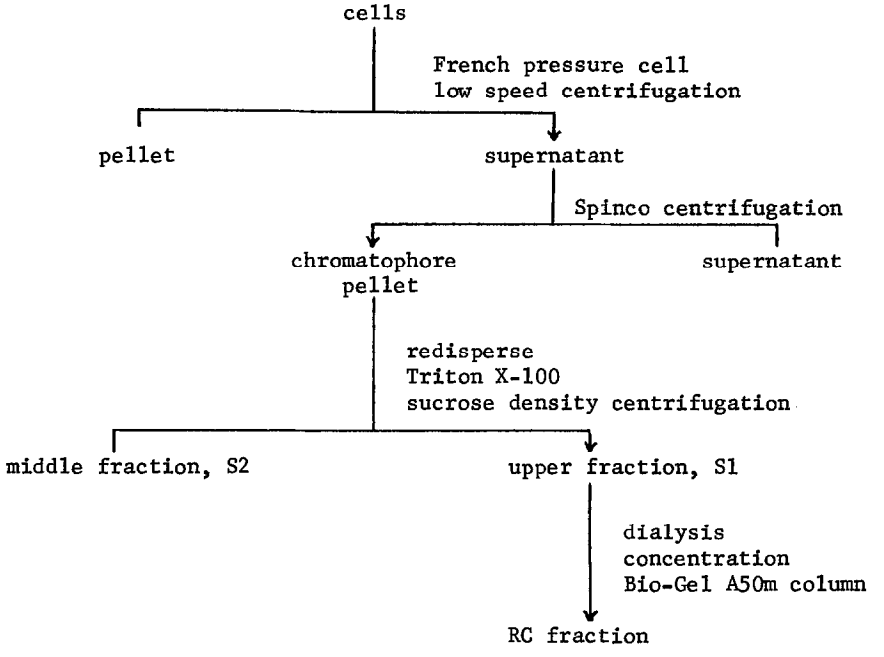
Figure 1

ISOLATION OF RC FRACTION



Absorption spectra were determined with a Cary model 14R recording spectrophotometer. Light-induced absorbance changes were determined by using the "IR-2" mode, in which the samples are exposed to intense white light. Concentrations of B870 (light-harvesting bacteriochlorophyll) and P870 were calculated by using their extinction coefficients of 127 $mM^{-1}cm^{-1}$ and 113 $mM^{-1}cm^{-1}$ respectively (Clayton, 1966).

The purification proceedure is shown diagramatically in Figure 1 and the bacteriochlorophyll composition of each fraction is listed in Table 1. All steps were carried out at 0^{o} to 10^{o} in 0.01 M Tris hydrochloride buffer, pH 7.5. Rps. spheroides was grown according to the method of Sistrom and Clayton (1964).

Cells were harvested by centrifugation, rinsed with buffer

Table 1

BACTERIOCHLOROPHYLL COMPOSITION

OF ISOLATED FRACTIONS

Fraction	B870 μmoles	P870 μmoles	$A_{280\ m\mu}:A_{870\ m\mu}$
Chromatophore	1.73	0.18	1.67
S1	0.00	0.05	----
S2	0.55	< 0.001	----
RC fraction	0.00	0.04	10.2

and broken by passage through a French pressure cell. The pellet which formed after centrifugation for 30 min at 20,000 x g was discarded. The chromatophore fraction was recovered from the supernatant by centrifugation for 60 min at 260,000 x g (60,000 rpm in the No. 65 rotor of a Spinco model L2-65 centrifuge) and this pellet was suspended in fresh buffer to give an absorbance of 50 at 870 mμ. Triton X-100 (10%, v/v), 0.3 volumes, was added slowly to the chromatophore suspension in an ice bath and stirring was continued for 60 min. Portions of this suspension, 4 ml, were layered onto discontinuous sucrose density gradients formed from 4 ml of 1.0 M sucrose and 4 ml of 0.6 M sucrose in 13.5 ml Spinco centrifuge tubes. Centrifugation for 90 min at 260,000 x g resulted in the separation of three bands.

The upper band, S1, remained in the supernatant layer above the 0.6 M sucrose. This fraction contained the reaction center bacteriochlorophylls, P800 and P870 (Table 1), and no B870. A small amount of solubilized bacteriochlorophyll in this fraction was removed during subsequent purification steps. A second band, S2, which layered above the 1.0 M sucrose contained B870; no

reversible light-induced absorbance changes due to the presence of P800 or P870 could be detected in this fraction. A third nearly colorless fraction formed a pellet at the bottom of the tube.

Fraction S1 was dialyzed for 24 hr against three 1-liter volumes of buffer and concentrated to 4.0 ml by pressure dialysis. The concentrated solution was chromatographed on a 2.2 x 90 cm Bio-Gel A50m column to remove a bacteriopheophytin-rich contaminant. In Figure 2 are spectra of the purified RC fraction in buffer which contained 1 mM dithiothreitol. The dithiothreitol was added to reduce P870 which had been isolated in the partially oxidized form. The final preparations appeared to be free of Triton X-100 because they did not foam when shaken. Triton X-100 also exhibits an absorption band near 225 mμ which was not detected in these

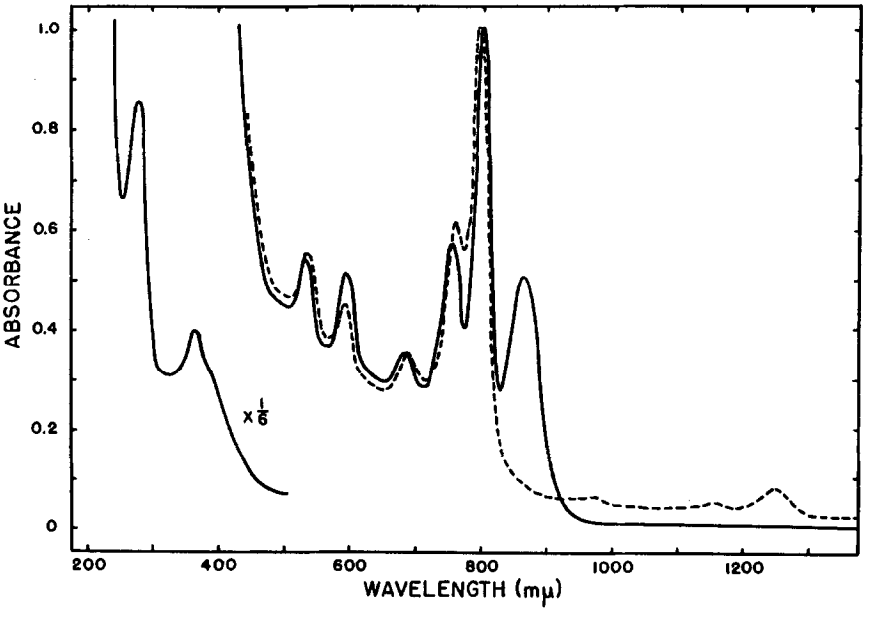


Fig. 2. Absorbance spectra of Reaction Center fraction in 0.01 M Tris hydrochloride buffer, pH 7.5, containing 0.001 M dithiothreitol. Spectra were determined in the "IR-1", ———, and "IR-2", -----, modes of the Cary model 14R recording spectrophotometer. In the "IR-2" mode the sample was exposed to undispersed white light.

preparations. Furthermore, no Triton X-100 was found in a diethyl ether extract from a freeze-dried preparation. The presence of some bacteriopheophytin is evidenced by the absorption bands near 530 and 760 mμ.

This proceedure is a simple method for obtaining reaction centers in a purified form. This preparation contained 1 μmole of P870/230 mg of protein as determined by the method of Lowry, et al. (1951). The low absorbance in the ultraviolet region makes it possible to measure absorbance changes at 280 mμ with a Cary recording spectrophotometer. Studies concerning the structure, reactivity and composition of these preparations are currently in progress.

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