A CAROTENOPROTEIN FROM CHROMATOPHORES OF RHODOSPIRILLUM RUBRUM

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SUMMARY

A carotenoprotein has been obtained by SDS-solubilization of Rhodospirillum rubrum chromatophores. It was then purified by $(NH_4)_2SO_4$ precipitation and Sephadex G-200 filtration. SDS-polyacrylamide gel electrophoresis revealed a single protein with a molecular weight of about 12,000. The absorption spectrum of the complex is entirely different from the usual three peaked carotenoid spectrum, it has only a major peak at 370 nm. However, after acetone extraction the spectrum of spirilloxanthin reappears. The fact that the carotenoid associates with a specific protein provides strong evidence that the complex originates from the chromatophores and is not a preparative artefact.

The existence of carotenoproteins in aquatic invertebrates and in non photosynthetic bacteria is well substantiated (1,2,3,4). Such is not the case, however, for photosynthetic organisms. β-carotene proteins have been obtained from spinach leaf chloroplasts (5,6), but their presence in vivo has been doubted and their existence attributed possibly to the preparation procedure (6).

A "carotenoid complex" extracted from chromatophores of Rhodospirillum rubrum by the combined action of Triton X-100 and pancreatin has been reported (7,8). It was not clear,

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however, whether this complex was proteinaceaous in nature or, again, whether it could not have arisen from the preparation procedure.

In this communication we will describe a method for the isolation of a complex involving spirilloxanthin and a specific protein.

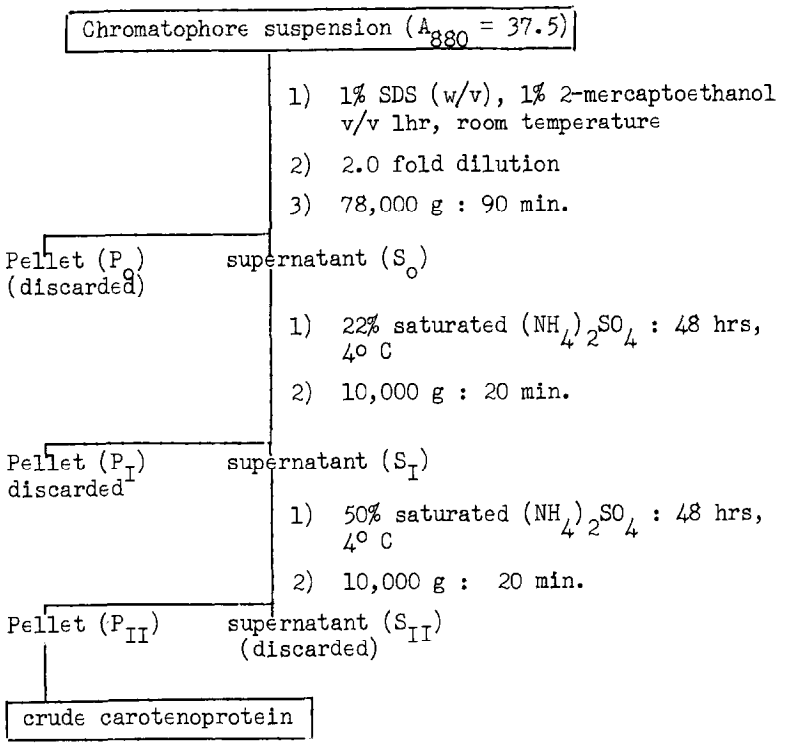
Rhodospirillum rubrum (American Type Culture Collection, strain no. 11170) was grown semi-anaerobically at 30° C under continuous illumination in the medium described by Cohen-Bazire et al (9). The cells were harvested by continuous centrifugation and washed with one half volume of cold 0.05 M Tris-HCl (pH 7.6) per volume of the initial culture. The chromatophores were obtained by grinding the bacteria with levigated alumina and subsequent differential centrifugation. The carotenoprotein was then extracted as follows by means of solubilization of the chromatophores with the anionic detergent sodium dodecyl sulfate (SDS).

The chromatophore suspension was brought to an absorbance of 37.5 at 880 nm and treated with 1 % SDS (w/v) and 1 % 2-mercaptoethanol (v/v) (Eastman Kodak Co.) in 0.05 M phosphate (pH 8) at room temperature for 60 min. Then an equal volume of cold buffer was added and the solution centrifuged at 78,000 g for 90 min. (rotor 30, Beckman L2-65 centrifuge). The supernatant S_0 was 22 % saturated with $(NH_4)_2SO_4$, allowed to stay in the cold for two days, and centrifuged at 10,000 g for 20 min. The carotenoprotein present in the supernatant S_I was precipitated by further addition of $(NH_4)_2SO_4$ so as to make the solution 50 % saturated, kept in the cold for another two days and centrifuged again at 10,000 g for 20 min. The red pellet P_{II} was dissolved in an amount of buffer (usually 10 mM

phosphate, pH 7.0) sufficient to obtain an absorbance value of 6 at 370 nm, the absorption peak wavelength. The preparation was then dialyzed for two days at room temperature against 10 mM phosphate buffer (pH 7.0) containing 0.01 % SDS and stored in the cold.

The isolation procedure of this crude preparation is outlined in the following diagram.

Diagram



This crude preparation has been purified by column filtration on Sephadex G-200 in 10 mM phosphate buffer (pH 7.0) containing 0.01 % SDS. The ratio of protein to spirilloxanthin content was measured in the crude and in the purified preparation. Protein was assayed by the method of Lowry et al (10) and spirilloxanthin by its absorption coefficient ($E^{1\%}_{1cm}$) of

2,470 at 510 nm in benzene. The average of ten determinations is given in the following table.

Table 1

	protein/spirilloxanthin mass ratio	mass of protein/mole spirilloxanthin
crude preparation	115	68,425
purified preparation	23.2	13,800

Polyacrylamide gel electrophoresis of the purified complex (solubilized in 1 % SDS, 1 % 2-mercaptoethanol) in the presence of 0.1 % SDS was carried out according to the method of Weber and Osborn (11). After staining the gel with Coomassie blue, a single band was revealed. This indicates the absence of major contaminating proteins (figure 1). According to its relative mobility, the protein is estimated to have a molecular weight of about 12,000, compatible with the value of 13,800 calculated for the minimal molecular weight of the purified preparation (based on the weight of protein containing one mole of spirilloxanthin).

The absorption spectrum of the purified complex is strikingly different from that of the carotenoid extracted from it (figure 2). Instead of the usual three peaked structure of carotenoids, the spectrum of the complex shows a single main peak at 370 nm and a minor one at 295 nm. The complex was extracted with acetone at room temperature and the extract transferred into diethylether, washed with distilled water and dried over anhydrous Na_2SO_4. The ether was then

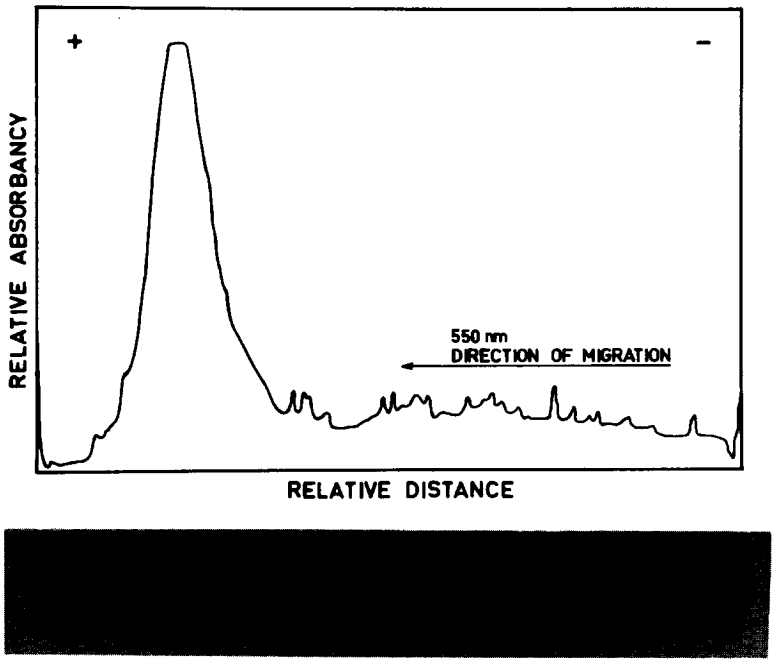


Figure 1 : SDS-Polyacrylamide gel electrophoresis of the carotenoprotein

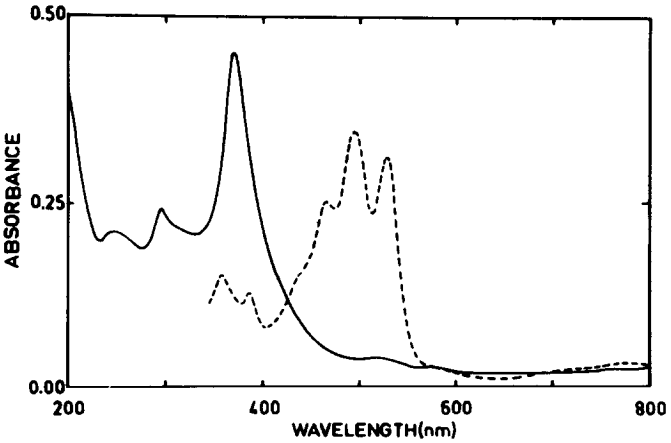


Figure 2 : Absorption spectrum of the carotenoprotein in 0.01 M phosphate, pH 7, in the presence of 0.01 % SDS, the protein content is 0.02 mg/ml. Also shown (dotted line) the spectrum of spirilloxanthin in petroleum ether after extraction from the complex with acetone.

evaporated under reduced pressure and the pigment dissolved in petroleum ether. The spectrum of this solution shown in figure 2 is typical of spirilloxanthin.

In view of the fact that spirilloxanthin has been shown to be linked to a specific protein, we think it unlikely that the complex described here is a preparative artefact. More probably the carotenoprotein is part of the chromatophore membrane structure and may contribute to its stabilization. The hypothesis that this is a membrane protein is reinforced by its hydrophobic nature indicated by its insolubility in the absence of added detergents.

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