PRELIMINARY NOTES

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Some optical properties of a "carotenoid complex" derived from *Rhodospirillum rubrum**

This is a preliminary report on the observation of a marked extrinsic optical activity of a "carotenoid complex" prepared from chromatophores of a photosynthetic bacterium, *Rhodospirillum rubrum*. The chromatophores, which were prepared from whole cells by sonic disruption, were acted upon by pancreatin (a crude mixture of pancreatic digestive enzymes) in the presence of the detergent Triton X-100 at 37° for 24 h (ref. 1). After sucrose density-gradient centrifugation, a brownish band containing practically all of the carotenoids can be separated. The material in the brown band has been designated a "carotenoid complex". In addition to the brown band, a blue and a green band containing mainly bacteriochlorophyll were also separated.

As reported previously[1], the major absorption band of the brown carotenoid complex has shifted toward a shorter wavelength from that usually observed for carotenoids in an organic solvent. A typical absorption spectrum of an aqueous suspension (in 0.1 M Tris buffer, pH 7.5) of the brown complex that was twice purified by sucrose density-gradient centrifugation and then dialyzed against water to remove Triton is shown in Fig. 1A. The optical-rotatory-dispersion (ORD) and circular-dichroism (CD) spectra of the same solution are shown in Fig. 1B.

The acetone solution of the brown complex at an identical concentration has the typical triple-peaked absorption spectrum in the visible region but no optical rotation, as shown by the dashed curves in Fig. 1. The absorption maxima of the acetone solution were at 373, 387, (466), 494 and 526 mμ. The maxima in the visible region appear to represent average values of the major carotenoid, spirilloxanthin, and that of some other carotenoids absorbing at slightly shorter wavelength, for instance, rhodovibrin[2]. The maxima in the ultraviolet region agree closely with those of spirilloxanthin. The absorption spectrum of the acetone solution of the complex is practically identical with that of the untreated chromatophores, indicating that enzymatic digestion had no apparent effect on the structure of the carotenoid molecules. Preliminary thin-layer chromatography showed that most of the carotenoids originally present in the *R. rubrum* chromatophores were detectable. The exact carotenoid composition requires further purification of the complex and pigment analysis.

As seen in Fig. 1A, the ultraviolet absorption spectrum of the brown complex has a major and a minor band at 368 and 296 mμ, respectively. The major absorption band has shifted from the shortest wavelength band of free carotenoids in acetone by 98 mμ; it also has a much narrower bandwidth. Both absorption maxima coincide with the maximum positive rotation, with Cotton effects observed on both sides of the absorption maximum. This can be more clearly seen from the double circular-dichroism spectrum shown in Fig. 1B. An approximate estimation, using typical

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Abbreviations: ORD spectrum, optical-rotatory-dispersion spectrum; CD spectrum, circular-dichroism spectrum.

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absorbance and molecular-weight values for carotenoids, yielded an exceedingly large molar rotation of approx. 10^7 degrees. The absorption spectrum of the complex indicates the absence of Triton X-100, and relatively little absorption in the aromatic amino acid region. The ORD spectrum shows little rotation in the 235 mμ region that may be attributable to an α-helical protein. However, the ultraviolet region of the CD spectrum appears to suggest an α-helical peptide structure[2].

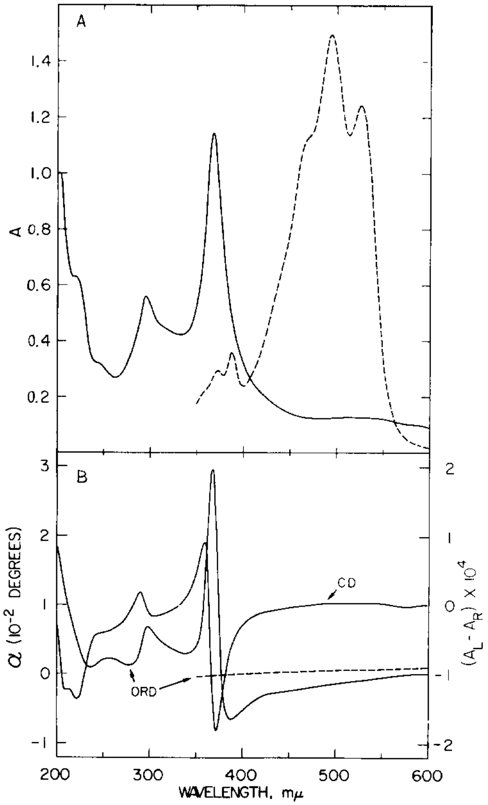

Fig. 1. Absorption spectra (A) and optical-rotatory-dispersion (ORD) and circular-dichroism (CD) spectra (B) of the "carotenoid complex" in an aqueous suspension (———) and in acetone (– – – – –).

Since the carotenoids in the chromatophores have an absorption spectrum similar to that exhibited in an organic solvent, and since the brown complex contains almost all the carotenoids originally present, the brown complex must have been formed as a result of enzymatic digestion in the presence of the detergent. It is likely that the carotenoids were removed from the lipoprotein matrix during the enzymatic digestion step. Under the experimental condition used, the free carotenoids may have reaggregated into a "complex" with the modified absorption spectrum and optical rotation. Preliminary ultracentrifugation and electron-microscopy experiments support this suggestion. Aggregates in the form of regular platelets have been observed by electron microscopy.

On the basis of the recently developed molecular exciton theory[3], the observed perturbation of the absorption spectrum of the pigment aggregate may be caused by an interaction arising from strongly coupled transition dipoles arranged in a card-pack ensemble. In the card-pack situation, the exciton wave function yields the highest-energy state, thus causing the blue shift. The narrowing of the bandwidth indicates that in the aggregate only the vibrationless electronic transition is allowed by the Franck-Condon principle. The narrow bandwidth could also be indicative of the homogeneity of aggregate sizes. The approximation of the blue-shifted peak position to the usual carotenoid "*cis*-peak" further suggests that the molecular arrangement in the aggregate could be such that the *cis*-peak transition moments are greatly enhanced. The acquired optical rotatory power by the pigment aggregate could conceivably be induced by the formation of a helix from the linear polymer[5], with the initiation occurring presumably at a local point of an α-helical polypeptide present in the digestive mixture.

To our knowledge, no aggregates of carotenoids derived from a photosynthetic organism have been reported to display such a large blue shift in their absorption spectrum. For instance, a β-carotene–protein complex isolated from spinach[6,7] shows a red shift and broadening of the triple-peaked absorption band and an appearance of a fourth band[7]. Aqueous suspensions of α- and β-carotene (crystals?) show only a broadening of the triple-peaked absorption band and an appearance of an additional band at a longer wavelength[8]. At the writing of this note, an aggregate of astaxanthin, the chromophore of the lobster shell pigment, crustacyanin, has just been reported[9]. The optical properties of the astaxanthin aggregate resemble closely those of the complex described in this note. Aqueous suspensions of astaxanthin aggregates show a large blue shift in its absorption maximum and also a markedly enhanced optical rotation. The spectral shifts in the yellow lobster pigment are believed to be caused by a similar type of interaction.

It should be pointed out, however, that while the present preparation procedure[1] always yields a complex with the large spectral shift toward the shorter wavelength, an optically active material has been obtained only part of the time. Further investigations on the nature of the acquired optical rotation and such other aspects as the exact condition for the formation of the carotenoid complex and their other physicochemical properties are in progress.

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