THE ORIGIN OF THE LONG WAVELENGTH ABSORPTION BANDS IN PURPLE BACTERIA

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Summary: From a study of the effect of the mild detergent octanoic acid on the circular dichroism of bacterial chromatophores it is deduced that the long wavelength absorption band of the bacteriochlorophyll seen *in vivo* at around 860 nm compared to the *in vitro* absorption around 770 nm is due to a specific interaction between the monomeric bacteriochlorophyll pi-electron system and certain functional groups in the protein with which it is associated in the biological system.

The long wavelength absorption of bacteriochlorophyll a (BChl) in organic solvents has a single near infrared band at 770 nm (Olson and Stanton, 1966). BChl also exhibits an orange absorption peak at 590 nm. The absorption spectra of purple bacteria all show an orange band at 590 nm and one to three peaks in the near infrared region around 800, 850 and 890 nm (Duysens, 1964). The exact band positions vary slightly from species to species. Since the beginning of research with photosynthetic bacteria, scientists have tried to explain this unusually large red shift of 30 to 120 nm, hoping to get some insight into the state of pigments in the photosynthetic apparatus. It seems that the state of the pigments is important for their function. Originally the various far red bands in purple bacteria were assigned to special bearer-pigment interactions (Wassink *et al.*, 1939; Duysens, 1952). Because solvent shifts failed to show more than 15 to 30 nm change of the red absorption peaks, the above interpretation was abandoned and different states of pigment aggregations were made responsible for the far red absorption bands. Aggregated forms of BChl (Krasnovsky, 1969) were able to mimick the absorption spectra of purple bacteria, except that the bandwidths were much larger than the *in vivo* ones.

In this communication we present some evidence that the large red shift is not due to aggregation but to a specific environment (probably amino acid side chain)-pigment interaction. The existence of such a strong interaction could be important for the explanation of some of the primary events in the reaction center, where the actual energy conversion takes place.

In a recent investigation (Steffen and Calvin, 1970) we have shown that low pH achieved with hydrochloric acid changes only slightly the absorption spectrum of the chromatophore suspension of Rhodopseudomonas spheroides mutant R-26. The BChl in vivo is protected against pheophytinization. On the other hand, low pH produced by fatty acids (e.g., octanoic acid, O.A.) exhibits a detergent effect. Fig. 1 demonstrates that O.A. affects the absorption of the chromatophore suspensions, normally having peaks at 590 and 860 nm (B590-860). At medium concentrations of O.A. and pH around 5, B590-860 is converted to a species absorbing at 590 and 770 nm, resembling the spectrum of BChl in vitro. It seems that the protein-pigment complex in the thylakoid membrane unfolds under the influence of O.A. and the pigments are exposed. At higher O.A. concentrations with pH below 5 the spectrum changes to one similar to bacteriopheophytin (BPh) (B530-760). Low pH is able to remove the Mg ion from the exposed BChl. Centrifugation and resuspension of chromatophores in new Tris buffer partially reverses the spectral change. B590-770 shifts back to B590-860. It is interesting to notice that B530-760 reverses to a pigment absorbing at 530 and 850 nm. This suggests that it is not a specific amino acid (e.g., histidine)-Mg^{++} interaction which is responsible for the big spectral shift from 770 nm to 860 nm.

It is known that freshly made and aged chromatophores behaved differently. The chromatophores used for the measurements of Figs. 1 and 2 were stored in concentrated suspension in 0.01 M Tris buffer, pH 7.5, at 4°C for 3 months. According to the absorption spectrum, there was no difference between stored and fresh chromatophore suspensions. The difference was in the need of a somewhat higher O.A. concentration to get the same effect in the absorption

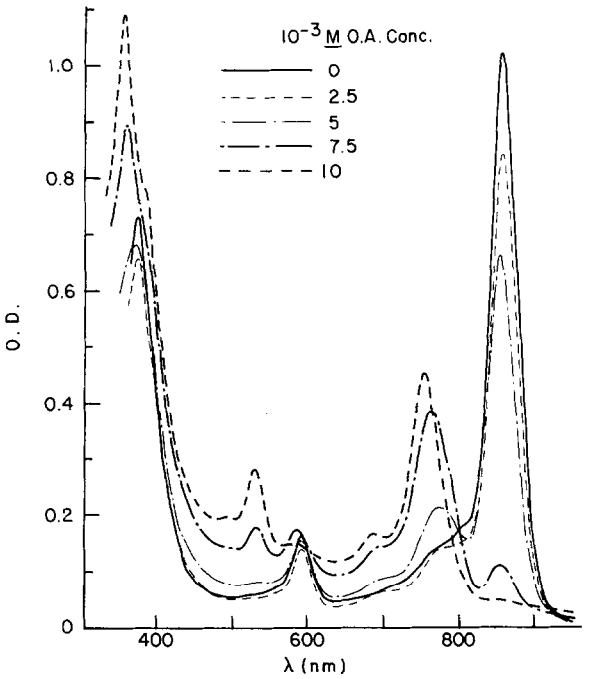

Fig. 1. Absorption spectra of $2.5 \cdot 10^{-3}$ (pH 6.5), $5 \cdot 10^{-3}$ (pH 5.2), $7.5 \cdot 10^{-3}$ (pH 4.85), and 10^{-2} M (pH 4.75) octanoic acid in equilibrium with Rhodopseudomonas spheroides mutant R-26 chromatophore suspension (5% ethanol).

spectrum, and according to the circular dichroism (CD) investigation, the O.A. breaks up the BChl-protein complex in a different way in the aged chromatophore suspension. The different circular dichroism spectra in Fig. 2 are labeled with "% change". This refers to the % decrease of the absorbance at 860 nm (as shown in Fig. 1). The main feature of the CD spectrum of the chromatophores alone (Fig. 2, 0% change) are the positive and negative bands at 850 and 880 respectively. This suggests that the bulk pigments are in the form of dimers incorporated into the protein carrier. The small bands at 810, 797 and 760 could have their origin in the reaction center (Sauer, Dratz and Coyne, 1968). By converting B590-860 to B590-770 or B530-760 with O.A., it seems that fatty acids break up dimers, because both the positive 850 and negative 880 band decrease. However, it can be seen that the 880 band disappears faster than the 850 band, indicating non-

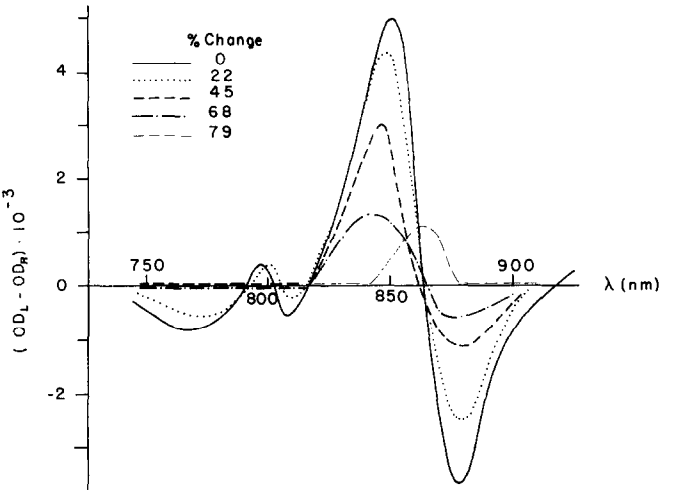

Fig. 2. Circular dichroism spectra of chromatophore suspensions titrated with octanoic acid. % change refers to Fig. 1 and is defined as % decrease of 860 nm absorption $100 \cdot \left(\frac{OD_0 \text{ M O.A.}(860) - OD_\chi \text{ M O.A.}(860)}{OD_0 \text{ M O.A.}(860)} \right)$

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degenerate interaction with the environment. At higher concentrations of O.A., a positive monomeric CD band appears at 863, the crossing point of the intact chromatophore CD spectrum and the main absorption peak of BChl *in vivo*. This result demonstrates that an unaggregated form of BChl still attached to the protein carrier can absorb at the *in vivo* band position of the chromatophores.

Additional information about a monomeric species absorbing around 860 nm emerges from our polarization measurements of the B590-860 fluorescence at 900 nm, excited in the 860 nm band (Steffen and Calvin, 1970). p-Values as high as 0.5 were observed with high O.A. concentrations, proving that no energy transfer takes place. If there were still some aggregation at 860 nm, energy transfer would occur, giving rise to depolarization effects.

From our results we conclude that the absorption peaks in purple bacteria correspond to specific interactions of the BChl π-electron system with amino acid groups of the protein carrier.

In case that such strong interactions are also present in the reaction center, they could be of importance in matching energy levels and modifying tunnel barriers between P870 and the acceptor site (according to Feher, 1970, probably an iron ion). There could be some conformational change in the protein of the reaction center by oxidizing P870, as was observed in the oxidation of cytochromes (for literature, see Chance et al., 1966).

We would like to mention an alternative explanation of the observed effect described in this communication. BPh-BChl complexes in vitro show also strong red shifted or absorption peaks (Katz, J. J., private communication, through Sauer, K.). However, it seems unlikely that such a complex would absorb exactly at in vivo absorption peak.

Acknowledgments

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