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MEDEDELINGEN VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN, NEDERLAND **63** (15) 1–8 (1963).

LIGHT SENSITIVITY AND EXTRACTABILITY IN PIGMENT-PROTEIN COMPLEXES OF THE PURPLE SULPHUR BACTERIUM *CHROMATIUM*, STRAIN D.

by

G. H. M. KRONENBERG

Laboratory of Plant Physiological Research, Agricultural University, Wageningen, Netherlands; 231st Communication, 86th Comm. on Photosynthesis

(Received 30.10.'63)

1. INTRODUCTION

The pigments of the photosynthetic bacteria are in submicroscopic particles, bacterial chromatophores. These are hydrophylic structures of considerable stability in which protein is supposed to be at the surface (1). There are several reasons to believe that pigments active in light absorption in photosynthesis are closely related to proteins. For instance, aqueous suspensions containing the chlorophyll or bacteriochlorophyll obtained by ultrasonics or by grinding show amphoteric motion in an electric field (2). Moreover, purple bacteria, probably dead cells, sometimes show spontaneous extrusion of a pigment complex containing bacteriochlorophyll and carotenoids, similar to that obtained by grinding of the cells.

Observations of shifts of absorption maxima upon extraction with organic solvents in purple bacteria also support the supposition of a pigment-protein bond *in vivo*. In several strains of purple bacteria, bacteriochlorophyll *in vivo* has two or three maxima in the near-infrared; they are different according to the species. In organic solvents, bacteriochlorophyll, regardless of the species from which it has been extracted, shows only one absorption peak at the same (shorter) wavelength. Other observations from earlier work include variation in relative height of the maxima for one and the same species, according to culture conditions, for instance light intensity during growth (2, 3).

The special type of complex formation between the pigment and its carrier, evidently determines the position of the near-infrared absorption maximum. WASSINK, KATZ and DORRESTEIN (3) concluded to the existence of different bacteriochlorophyll-protein complexes in mutually variable amounts, presumably present in each cell. Each of the maxima of the *in vivo* near-infrared absorption spectrum, represents one of the pigment-protein complexes. This conclusion has received considerable support from DUYSENS' observation (4) that energy transfer exists between the entities represented by each peak.

Fig. 1 shows the absorption spectrum of *Chromatium*, strain D. It has 3 absorption maxima in the near-infrared, at 800, 850 and 890 mμ respectively.

The absorption spectrum of an aqueous extract, obtained by grinding with carborundum or ultrasonic treatment is the same as that of cells, however, with less scattering.

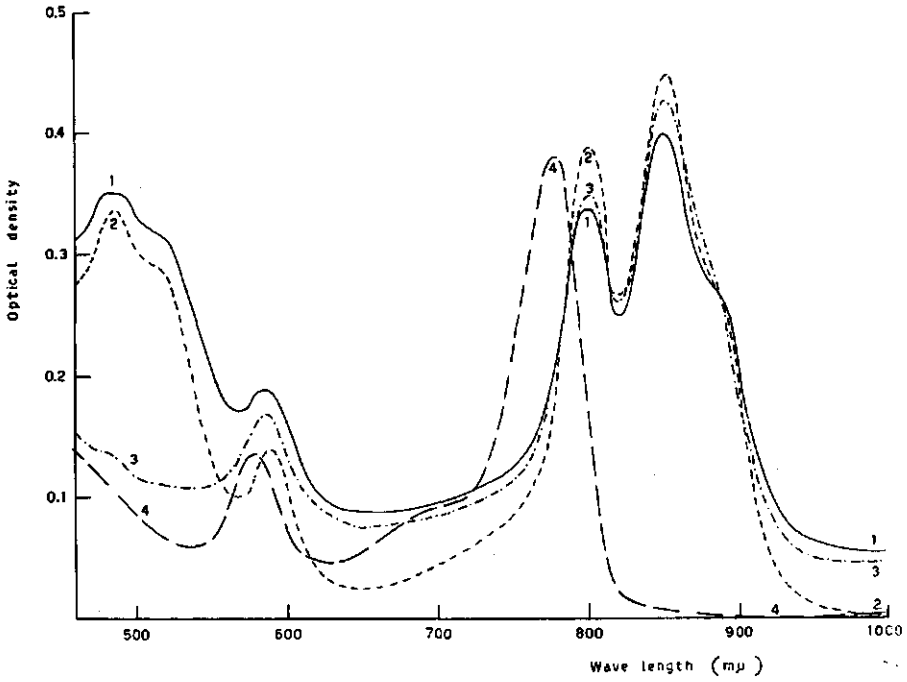

FIG. 1. The absorption spectra of *Chromatium*, strain D:
(1) cells grown on L-malate-thiosulphate medium;
(2) aqueous extract of 1 obtained by grinding;
(3) carotenoid-deficient cells, obtained by growth on L-malate-thiosulphate medium in the presence of 20 mgm DPA/liter;
(4) alcoholic extract of both 1 and 3.

As already remarked, organic extracts have a single absorption maximum in this region of the spectrum, shifted to shorter wavelength, the exact location depending on the solvent. Carotenoid-deficient *Chromatium* cultures can be obtained in the presence of diphenylamine (DPA) according to GOODWIN and OSMAN (5, 6). The suppression of the synthesis of coloured carotenoids is shown by lower absorption in the 450 to 550 mμ region of the spectrum. These cells are blue-green instead of purple, but the near-infrared absorption spectrum is the same as that of normal cells, except that the spectra of green cells and their aqueous extracts have a somewhat decreased 890 shoulder (7).

The main target of our study is to obtain a separation of the different pigment-protein complexes; for this reason a further characterization of these complexes and exploration of their properties is attempted (8). The present paper deals with differential effects of light and oxygen on the near-infrared

absorption maxima and a comparison of the results with the extractability of the pigment from the different complexes, represented by these maxima.

The effect of light and oxygen was investigated both in normal and carotenoid-deficient cells and their aqueous extracts, in order to see whether also in our strain carotenoid-deficient cells were more sensitive to photo-oxidation as is found by STANIER *c.s.* in *Rhodopseudomonas spheroides* (9, 10) and in *Rhodospirillum rubrum* (11).

2. EXPERIMENTS

a) The effect of light and oxygen

Samples of bacterial suspension or aqueous bacterial extract in phosphate buffer of pH 8.0, were taken and the absorption spectra were measured in a handoperated Beckman DU spectrophotometer after different periods of treatment. In dark, bleaching of both carotenoid-deficient and normal cells and their aqueous extracts, was negligible in an atmosphere with or without oxygen, for 5 hours. However, when white light and oxygen were applied together, strong bleaching took place. The light was obtained from a Tungsten lamp providing an intensity of about, 60,000 ergs/cm² sec., and air was bubbling through the bacterial suspension; the temperature was 15°C.

The effect on normal red cells was a rapid bleaching of the 850 mμ peak, while the 800 mμ peak during the initial period of exposure was almost constant, and sometimes even increased. In the course of time, the 850 mμ peak which usually is highest, decreased to far below the 800 mμ peak. Then the 800 mμ peak also decreased somewhat but the decrease of the 850 mμ peak is always much stronger. It must be assumed that the 890 mμ peak also

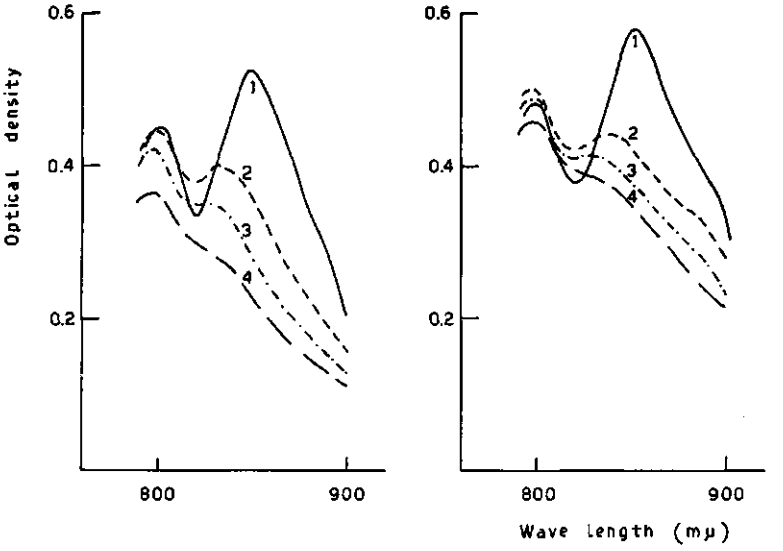

FIG. 2. Effect of light and oxygen on the absorption spectra of carotenoid-deficient cells (left), and normal cells (right) of *Chromatium*, strain D. The spectra after 0, 1½, 3 and 5 hours respectively (1–4). White light at an intensity of 60,000 ergs/cm² sec.; suspension continually flushed with air.

decreases rapidly, since even its expression as a shoulder on the 850 mμ slope disappears already early in the reaction sequence.

When carotenoid-deficient cells from cultures of the same age as the normal ones, were exposed to light and oxygen, the bleaching pattern was somewhat different. Both the 850 and 800 mμ peaks strongly decreased from the beginning of the experiment, but again the 850 mμ peak was affected fastest. Fig. 2 shows the bleaching of the spectra of normal and carotenoid-deficient cells. Samples were taken during 5 hours at time intervals of 30 minutes. For the sake of clarity, only four spectra have been presented. The difference in the belaching pattern between the normal and carotenoid-deficient cells is shown in figure 3. The combined action of light and oxygen on the aqueous

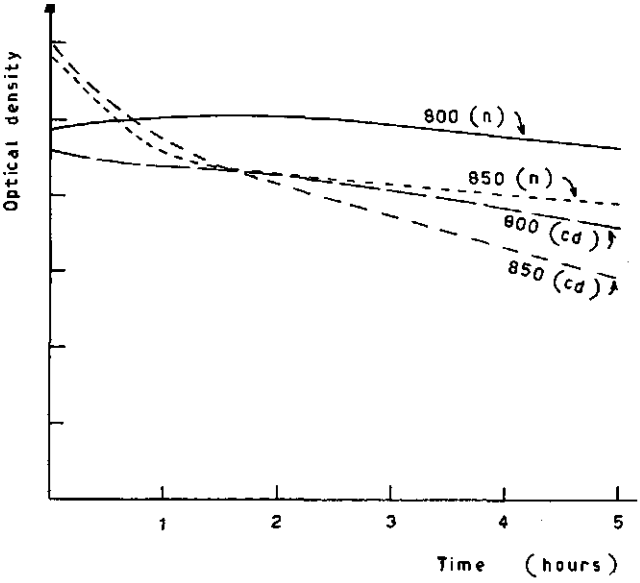

FIG. 3. Effect of light and oxygen on the absorption spectrum of *Chromatium*, strain D. Decrease of the 800 and 850 mμ absorption peaks of normal (n) and carotenoid-deficient (cd) cells. White light at an intensity of 60,000 ergs/cm² sec.; suspension continually flushed with air.

extracts of both carotenoid-deficient and normal cells, gave the same picture as the intact cells. Comparison of white light effects in normal and carotenoid-deficient cells is not devoid of difficulties, since bacteriochlorophyll in suspensions of purple bacteria is of necessity in part shielded by carotenoids. Therefore, the experiments have been repeated with red light (containing near-infrared also) in which carotenoid absorption is negligible. It was somewhat surprising, that preliminary results are very much the same as those in white light. In red light the 850 mμ peak also disappears more rapidly than the 800 mμ peak. Moreover, like in white light, bleaching of both peaks in carotenoid-deficient cells was somewhat more rapid than in normal cells.

In bleaching experiments it is important to use samples of cell suspensions or aqueous extracts immediately after their preparation, owing to the fact that bleaching of cells and extracts kept in the refrigerator for several months

is much slower than bleaching of fresh material. This was also found earlier for *Rhodopseudomonas spheroides* (12).

Another point to pay attention to is the anti-oxidative action of DPA. One must be sure that the carotenoid-deficient cells grown in the presence of DPA are free of this compound in bleaching experiments. DPA had to be washed out before use of the cells, otherwise it is possible that normally more sensitive green cells are bleached more slowly than normal cells, owing to the presence of DPA.

b) Extractability of pigment from the various bacteriochlorophyll complexes

Chromatium cells were extracted during a fixed period by mixtures of organic solvents in different concentrations in water. Hydrogen sulphide was added to prevent photo-oxidation of bacteriochlorophyll. Aside of this, extractions were made with a fixed concentration of organic solvent, during different periods. The absorption spectra of the cells were determined before and after the extraction, and also the spectrum of the organic extract was measured. Different alcohols, *e.g.* ethanol, propanols and butanols, ketones, *e.g.* acetone and methyl-isobutyl-ketone, and some other solvents were applied.

The general tendency was that increase in duration of extraction caused a rapid decrease of absorption in the 850–890 mμ part of the spectrum, while the 800 mμ peak decreased more slowly. Thus, the 850 mμ peak, usually higher in the cells, gradually became lower than the 800 mμ one during the extraction. Thereafter, the proportion of these two peaks remained almost constant during the further decrease of total bacteriochlorophyll absorption in the cell residue (fig. 4).

A similar picture resulted in the case of a fixed duration of exposure and increasing concentrations of the organic agent.

In both cases, the absorption maximum of free bacteriochlorophyll in the supernatant increased proportional to the decrease of bacteriochlorophyll content of the cellular residue as was to be expected.

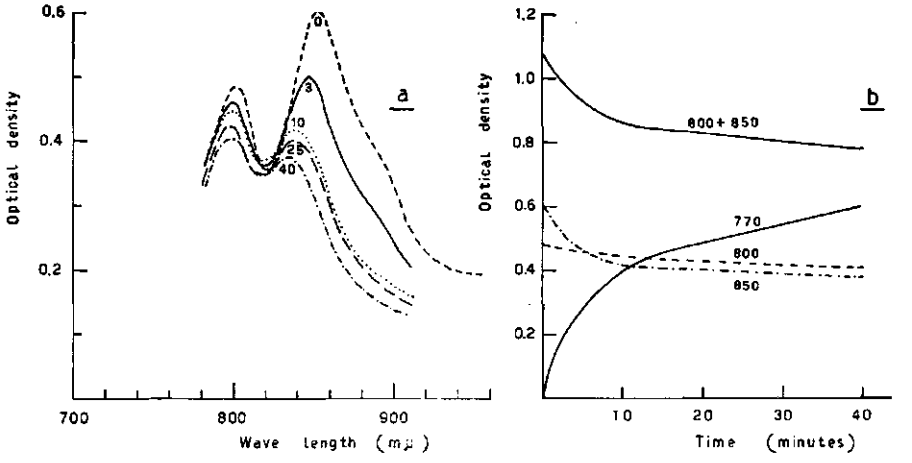

FIG. 4. Extractability of harvested cells. Extraction with 60% (v/v) tert. butanol in water during 0, 3, 10, 25, and 40 minutes (a). Decrease of 800 and 850 mμ absorption peaks and increase of extinction at 775 mμ in the spectrum of the aqueous-organic extract (b).

We have also looked for differences in extraction between normally grown purple cells of *Chromatium* and blue-green cells of the same age, grown in the presence of DPA. Preliminary results point to a difference in extractability between the two types. The green cells loose their bacterioclorophyll much faster than the red ones, independent of the presence of DPA.

Also in green cells we find a rapid decrease of the 850–890 mμ absorption, simultaneous with a slower decrease of the 800 mμ peak, as in normal cells. Total extraction of bacteriochlorophyll from carotenoid-deficient cells from which DPA is removed by repeated washings with phosphate buffer proceeded much faster than extraction from normal cells treated in the same way (fig. 5).

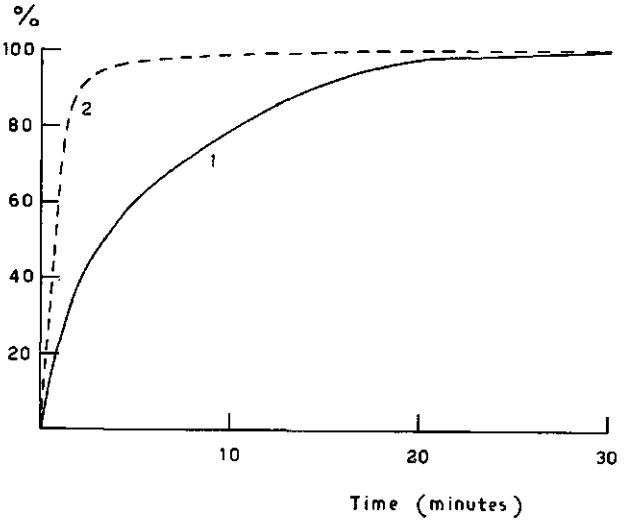

FIG. 5. Extraction with 80% (v/v) ethanol of normal (1) and carotenoid-deficient (2) *Chromatium* cells. The amount of bacteriochlorophyll extracted as percentage of total amount of bacteriochlorophyll present in the sample.

3. DISCUSSION

The results of bleaching experiments obtained until now, show stronger bleaching of the complexes absorbing at longer wavelengths, as was found earlier for normal *Chromatium*, strain D (13) and also stronger bleaching of bacteriochlorophyll in carotenoid-deficient cells. This is in agreement with STANIER's results with carotenoid-deficient *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides* (9, 10, 11).

It should be pointed out, however, that the relation between lack of carotenoids and the greater photosensitivity of the blue-green cells, does not necessarily demonstrate a protective action of the carotenoids. The carotenoid-deficient cells might be different in other, not directly observable properties, resulting in increased photosensitivity. The absence of carotenoids then, is only of secondary importance. If the carotenoids are directly involved in protection of bacteriochlorophyll against photo-oxidation, this should be by way

of „dark-chemical" reactions as the results obtained in red light demonstrate. The experiments in red light leave open the possibility that in light, absorbed also by carotenoids, an additional, directly photochemical protection of bacteriochlorophyll by carotenoids exists.

The pattern of the decrease in absorption by extraction with various solvents, both in normal and green cells is comparable with the decrease in absorption by the action of light and oxygen, as observed in carotenoid-deficient cells. The 800 and 850 mμ peaks both disappear from the beginning of the exposure, the 850 mμ peak first, being the most sensitive, while soon the rate of disappearance of both peaks becomes of the same order of magnitude. Our experiments all concur in demonstrating a greater sensitivity of the complexes, absorbing at the longest wavelengths. This may be due to differences in the proteins themselves, to which the pigment is bound, or to differences in the nature of the bonds of bacteriochlorophyll to the protein. The greater sensitivity of the 850 mμ peak as compared to the 800 mμ one, points to a closer connection between pigment and protein bearer in the 800 mμ peak. The 850 mμ material is closer to free bacteriochlorophyll in several respects, for instance in its greater sensitivity to light and solvents. A speculation in this connection may be, to assume a closer connection of bacteriochlorophyll with lipids in the 850 mμ complex, approaching conditions in solution. It then remains curious however, that the shift of the absorption peak as compared with true solutions is so much greater than in the 800 mμ complex. Also differences in the position of the complexes in the chromatophore may influence their sensitivity towards different agents. One complex can be situated more at the surface of the chromatophore and thus be more easily attacked than another which is more inside the chromatophore.

4. Summary

The present note reports on effects of light and oxygen on the near-infrared absorption maxima of *Chromatium*, strain D in comparison with the extractability of the pigment from the various complexes.

In normally grown cells the 890 and 850 mμ maxima were found distinctly more sensitive than the 800 mμ peak towards light and oxygen, as well as to several types of solvents.

Carotenoid-deficient cells were found somewhat more sensitive to white light than normal ones, with about – though not quite – the same relation between sensitivity of the various peaks. The same holds for solvents tested. Interestingly, in red light (including near-infrared), not absorbed by carotenoids, the same results were obtained both in normal and carotenoid-deficient cells (preliminary observations). This points to a chemical or structural rather than photochemical "protection", if any.

Acknowledgement

The investigation reported in this paper was enabled by Grant AF EOAR 62–30, of the Office of Airospace Research, of the U.S. Air Force; the author is grateful for having been able to use the facilities provided by this support.

Particular thanks are due to Professor E. C. Wassink for his collaboration and stimulating suggestions. I also like to thank Miss J. W. J. M. Jansen, Miss W. Vos, Mr. R. v. Beek and Mr. D. Stedelaar for technical assistance.

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Added in proof

The main contents of this paper were discussed at meetings of the Biochemical Society and the "Nederl. Ver. v. Biochemie", Leiden 16–17 May, 1963 (Summary in: Chem. Weekblad **59**, 593 (1963)), and of the Society for Experimental Biology, at Oxford, U.K., 16–19 July, 1963.

After the completion of our manuscript, we found that C. BRIL, Biochem. Biophys. Acta **66**, 50–60 (1963) has studied photo-oxidation in bacterial chromatophores in the presence and absence of detergents; under these conditions the green cell type also was more sensitive than the red one, and BRIL's explanation goes along the same line as ours.

In an article, quite recently received as a reprint, entitled '13. Comparative structure and activities of the microbial photosynthetic apparatus', pp. 223–241 (booktitle not available), R. C. FULLER mentions observations on the near-infrared absorption spectra of *Chromatium* at high and low light intensities. In malate cultures the results appear very similar to those communicated by WASSINK *et al.* 25 years ago, see ref. 2,3 of the present paper (not quoted by FULLER). The changes observed in CO_2-cultures at first sight seem more complicated, however with a similar tendency.

FULLER observed an increased (pigment and) lipid content in low light intensity-grown cells. It remains to be seen how this pertains to the suggestion, derived at p. 7 of our paper, of a closer contact of lipids with the 850 mμ peak, referring *e.g.* to its greater sensitivity to organic solvents.

The present author has recently studied the relations between light intensity and absorption spectrum in greater detail, in normal and carotenoid-deficient cells. The earlier results are confirmed (described in WASSINK and KRONENBERG, Technical Report AF EOAR Grant 62–30, Biosciences, completed 1.1.64 (see also ref. 8) and in preparation for publication elsewhere).