# LIGHT-INDUCED SPECTRAL SHIFTS IN BACTERIOCHLOROPHYLL AND CAROTENOID ABSORPTION IN PURPLE BACTERIA

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Light-induced absorbancy changes in the near-infrared wavelength region in intact cells and cell free preparations of photosynthetic bacteria have been object of several investigations (Duysens, 1952; Duysens et al., 1956; Arnold and Clayton, 1960; Clayton, 1962; Vredenberg and Duysens, 1963; Sybesma and Vredenberg, 1963, 1964; Kuntz et al., 1964). An absorption decrease around 870-890 mμ in purple bacteria has been attributed to the oxidation of a reaction center P 890 upon illumination.

Recently Clayton (1963) reported in Rhodopseudomonas spheroides a different type of infrared absorption changes. These changes, which are not observed in Rhodospirillum rubrum (e.g. Duysens et al., 1956), could not be attributed to the oxidation of P 890, because of their kinetics and sign at 890 mμ. This paper gives the results of a closer study of the characteristics of these changes in various species of sulphur and non sulphur purple bacteria. In contradistinction to Clayton we conclude that they are not caused by a primary light reaction.

## METHODS

Athiorhodaceae were grown in a malate containing medium after Hutner, as reported earlier for Rhodospirillum rubrum (Amesz, 1963), Chromatium in the medium of Hendley (1955). The apparatus for measuring absorbancy changes was the same as used in other experiments in this laboratory (see Amesz, 1964). The bacteria were resuspensed in fresh growth medium. The absorbancy in the measuring vessels was about 0.8 - 1.0 at the highest infrared absorption maximum.

## RESULTS AND INTERPRETATION

We confirmed Clayton's observation of an absorbancy increase around 880 mμ in Rhodopseudomonas spheroides upon actinic illumination of a moderate intensity. The absorption difference spectrum of the green mutant EMS 65 (obtained by courtesy of Prof.Dr. R.Y. Stanier) under these conditions, shown in Fig. 1, is similar to that measured by Clayton (1963, Fig. 4). For actinic light of 600 mμ the shape of the difference spectrum was independent of the intensity from $2 \cdot 10^{-10}$ to $15 \cdot 10^{-10}$ einstein/(sec $cm^2$). This suggests that the changes at different wavelengths are due to one reaction and that the dip in the difference spectrum at 870 mμ is not caused by the oxidation of P 890. Above $15 \cdot 10^{-10}$ einstein/(sec $cm^2$) interfering absorbancy changes due to P 890 were observed. The mutant contained a considerable amount of carotenoids and the infrared absorption spectrum was similar to that of the wild strain.

The absorption difference spectrum of the wild strain of Rhodopseudomonas spheroides, measured under about the same experimental conditions was similar to that of the mutant (Fig. 1). Except for a slow irreversible increase at wavelengths above 840 mμ upon

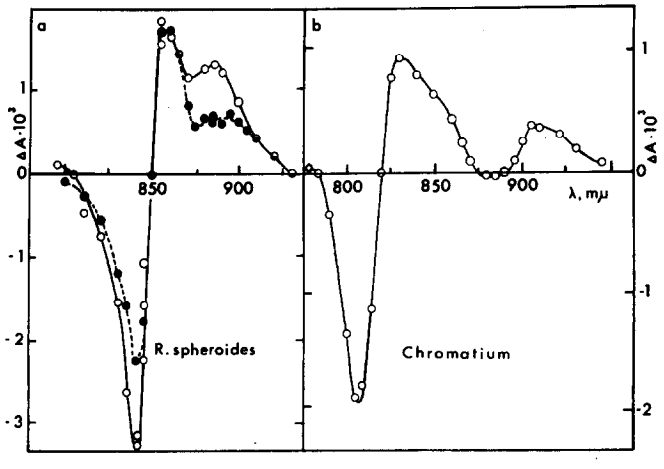


Fig. 1. Absorbancy difference spectra light minus dark of (a) Rhodopseudomonas spheroides wild strain (solid circles) and strain EMS 65 (open circles) at 2°C. Illumination was a band of 580 - 620 mμ of intensity $15 \cdot 10^{-10}$ einstein/(sec $cm^2$). (b) Chromatium, strain D, at 20°. The illumination was a band around 400 - 450 mμ of intensity $6 \cdot 10^{-10}$ einstein/(sec $cm^2$).

prolonged illumination, the absorbancy changes were reversed within a few seconds. The shape of the spectra and the approximate equality of the positive and negative areas suggest shifts of the absorptionbands of bacteriochlorophyll molecules absorbing at about 840-850 mμ to around 855 mμ and 880 - 900 mμ.

Chromatium, strain D, gave a different spectrum (Fig. 1). In this species a bacteriochlorophyll band at 810 - 820 mμ appears to be shifted. Absorbancy changes of the type found in the species mentioned above were not observed in Rhodospirillum rubrum, strains 1 and 4 ( in agreement with earlier results) and in Rhodopseudomonas palustris.

Action of light of different wavelengths: The relative efficiency of quanta absorbed at 593, 880 and 905 mμ to bring about the absorbancy decrease at 810 mμ in Chromatium was found to be about the same: 1.00, 1.13 and 0.96 respectively. This indicates that quanta absorbed by the various bacteriochlorophyll types are about equally efficient, as was also found for other light-induced processes in purple bacteria (Duysens, 1952; Amesz, 1963; Vredenberg, in preparation) and that the changes are activated by the same pigment system.

Effect of temperature and chemical agents: The absorbancy changes were not observed in Chromatium at -170°C and -50°C.

Phenylmercuric acetate (PMA) strongly enhanced the magnitude of the absorbancy changes in Rhodopseudomonas spheroides and Chromatium (see Fig. 2), apparently by decreasing the rate of the reversion of the reaction upon darkening.

In the presence of $5 \cdot 10^{-5}$ M potassium ferricyanide and $15 \cdot 10^{-5}$ M dichlorophenol indophenol (DPIP), the light-off reaction was accelerated and the steady-state lowered in PMA-treated Rhodopseudomonas. Addition of ascorbate ($6 \cdot 10^{-5}$ M) and DPIP ($1.5 \cdot 10^{-5}$ M) to PMA-treated cells had rather the opposite effect (Fig. 2). This suggests that the light-induced infrared absorbancy changes reflect the reduction of a cellular compound, a suggestion also made by Clayton (1963) from similar results with chromatophores.

Light-induced changes in carotenoid absorption: For Rhodopseudomonas spheroides several authors (e.g. Smith and Ramirez, 1960, and Nishimura and Chance, 1963) have reported light-induced absorbancy changes in the carotenoid region (440 - 550 mμ), which were stimulated by PMA. We found that the changes at 490 and 472 mμ were about equally stimulated by PMA as those at 840 mμ (Fig. 2).

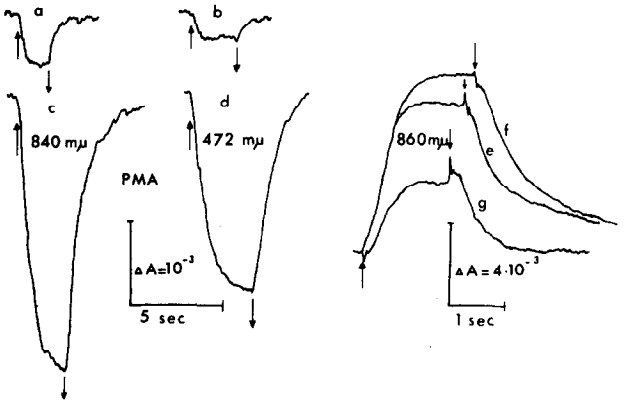


Fig. 2. Absorbancy changes in Rhodopseudomonas spheroides, wild strain at 22°C. Upward and downward pointing arrows mark the beginning and the end of an illumination period. Left hand part of the figure: kinetics at 840 mμ (a) and 472 mμ (b) upon illumination with light of 583 mμ of an intensity of $2.2 \cdot 10^{-10}$ einstein/(sec $cm^2$). Tracings (c) and (d): the same after addition of PMA ($5 \cdot 10^{-5}$ M). Right hand side of the figure: kinetics at 860 mμ in the presence of $5 \cdot 10^{-5}$ M PMA at $10 \cdot 10^{-10}$ einstein/(sec $cm^2$); (e) without further additions, (f) in the presence of $6 \cdot 10^{-5}$ M ascorbate and $1.5 \cdot 10^{-5}$ M DPIP and (g) in the presence of $5 \cdot 10^{-5}$ M ferricyanide and $1.5 \cdot 10^{-5}$ M DPIP. Note the different scales for the two parts of the figure.

In the presence of PMA the kinetics and the saturating intensity were the same at the three wavelengths. This suggests that the changes in carotenoid and bacteriochlorophyll absorption are caused by the same phenomenon. The kinetics in the absence of PMA, and the degree of stimulation by PMA were somewhat different at the three wavelengths, indicating the contribution by other absorbing substances at one or more wavelengths. The infrared as well as the carotenoid absorption changes were most pronounced under anaerobic conditions. In Chromatium the infrared absorbancy changes were not accompanied by changes in carotenoid absorption, neither in the presence, nor in the absence of PMA.

## DISCUSSION

At present there is only fragmentary evidence concerning the nature of the reaction which gives rise to the observed absorbancy changes. There are indications that they are due to a light-induced reduction of a cellular compound. But more evidence is certainly needed, in view of the report by Smith and Ramirez

(1960) that the carotenoid absorption changes in Rhodopseudomonas spheroides were also brought about by oxygen.

The absence of the infrared changes in both strains of Rhodospirillum rubrum and in Rhodopseudomonas palustris contradicts the assumption that the changes are due to a chemical reaction of a universally occurring pigment. Moreover, it seems unlikely that the shift of the absorption spectra is caused by a chemical reaction in the porphyrin ring of the bacteriochlorophyll molecule. It may be due to a change of its chemical environment, e.g. by a conformational change in the lipoprotein adjacent to the bacteriochlorophyll. The same can be said of the concomittant changes in carotenoid absorption in Rhodopseudomonas spheroides (cf. Smith and Ramirez, 1960). It is possible that the reaction that causes the changes occurs in all purple bacteria, but that for some species the reaction is not accompanied by absorbancy changes because of a different subcellular structure.

The rapidity of the changes at rather low light intensity and the relative activity of actinic light of different wavelengths suggest that they are caused by a photosynthetic reaction, activated by the reaction center P 890. The observation that the changes are absent at -50°C and the fact that, in the presence of PMA, the light intensity necessary to saturate the changes was much lower than for P 890 oxidation, indicate that the absorbancy changes are not caused by a primary reaction, as assumed by Clayton (1963).

## REFERENCES

Amesz, J., Biochim. Biophys. Acta, 66, 22 (1963).
Amesz, J., Thesis University of Leiden, 1964.
Arnold, W., and Clayton, R.K., Proc. Natl. Acad. Sci. U.S., 46, 769 (1960).
Clayton, R.K., Photochem. Photobiol., 1, 201, 305 (1962).
Clayton, R.K., Proc. Natl. Acad. Sci. U.S., 50, 583 (1963).
Duysens, L.N.M., Thesis University of Utrecht, 1952.
Duysens, L.N.M., Huiskamp, W.J., Vos, J.J., and van der Hart, J.M., Biochim. Biophys. Acta, 19, 188 (1956).
Hendley, D.D., J. Bacteriol., 70, 625 (1955).
Kuntz Jr., I.D., Loach, P.A., and Calvin, M., Biophys. J., 4, 227 (1964).
Nishimura, M., and Chance, B., Biochim. Biophys. Acta, 66, 1 (1963).
Smith, L., and Ramirez, J., J. Biol. Chem., 235, 219 (1960).
Sybesma, C., and Vredenberg, W.J., Biochim. Biophys. Acta, 75, 439 (1963).
Sybesma, C., and Vredenberg, W.J., Biochim. Biophys. Acta, 88, 205 (1964).
Vredenberg, W.J., and Duysens, L.N.M., Nature, 197, 355 (1963).