SC 2350

Evidence for a reaction center P 840 in the green photosynthetic bacterium *Chloropseudomonas ethylicum*

Light-induced reversible changes have been observed in the absorption spectra of many photosynthetic organisms. One of the changes occurring in the infrared region in purple bacteria is a light-induced bleaching of a pigment which is assumed to be a reaction center where a primary photochemical reaction of photosynthesis takes place. It has been shown[1] that in the purple bacterium *Rhodospirillum rubrum* the light-induced bleaching at 890 mμ (presumably the photo-oxidation of a pigment which is called P 890 (refs. 1–3)) is quantitatively correlated with an increase of the fluorescence yield of bacteriochlorophyll. These experiments yield strong evidence that P 890 is a reaction center. We wish to report light-induced changes in the far-red absorption spectrum of the green photosynthetic bacterium *Chloropseudomonas ethylicum* and the correlation between these changes and an increase of the fluorescence yield of chlorophyll-770 (see below). A maximum decrease in absorption appears to occur at about 840 mμ, presumably due to the bleaching of a pigment which we will call P 840.

Cps. ethylicum, like the other green photosynthetic bacteria, contains in addition to the main chlorophyll, chlorobium chlorophyll (bacterioviridin), a small amount of another chlorophyll species with striking resemblances to bacteriochlorophyll, which was called chlorophyll-770 (refs. 4, 5). In intact bacteria this chlorophyll is bound to cell constituents and the complex has an infrared absorption peak at 809 mμ. We call this complex B 810, in analogy with bacteriochlorophyll types B 800, B 850 and B 890 in purple bacteria[6]. It has been shown that there is a substantial energy transfer from chlorobium chlorophyll to B 810 (ref. 7) and that light absorbed exclusively by B 810 is effective in oxidizing a *c*-type cytochrome[8]. It was found also that at high intensities of the exciting light, when photosynthesis is saturated, the fluorescence yield of B 810 is higher than at lower intensities, while the fluorescence yield of chlorobium chlorophyll remains constant in both intensity ranges[7,8]. These data suggest that excitation energy is transferred from chlorobium chlorophyll to B 810 and from B 810 to the primary photochemical reaction of photosynthesis.

Cps. ethylicum strain 2K was grown in the light in a medium containing 0.1% ethanol[9]. Whole cells suspended in growth medium and cells resuspended in water were used for the experiments. The measurements were carried out at 2°. The apparatus used was the split beam differential spectrophotometer shortly described previously[10]. Actinic light of 436 mμ for inducing changes in absorbancy and fluorescence was provided by a d.c.-fed slide projector with filters. The fluorescence yield was measured with an exciting beam of modulated light of 436 mμ at a constant intensity, which was too low to cause changes in absorbancy or fluorescence. A filter combination in front of the photomultiplier was used to select the wavelength of fluorescence. Since the apparatus was sensitive only for modulated light the fluorescence induced by the actinic light does not cause a deflection as a function of time.

The absorption difference spectrum of whole cells suspended in water is given in Fig. A preliminary scan of the spectrum of absorbancy changes from 400 mμ up revealed no changes in addition to those found previously[8], which were attributed

Abbreviation: PMA, phenylmercuric acetate.

to the light-induced oxidation of cytochrome. The light-induced decrease in absorbancy at 840 mμ was found to correlate with a light-induced increase of the fluorescence yield at 838 mμ, at which wavelength presumably all fluorescence is due to B 810 (ref. 7). No appreciable change in fluorescence was found at 764 mμ which is near the wavelength of maximum fluorescence of chlorobium chlorophyll[7]. This is in agreement with previous experiments[7,8]. When $2 \cdot 10^{-5}$ M PMA was added to the cells, photobleaching of P 840 still occurred while no oxidation of cytochrome (measured as the change in absorbancy at 423 mμ) could be demonstrated. The regeneration of P 840 in the dark, however, was substantially inhibited.

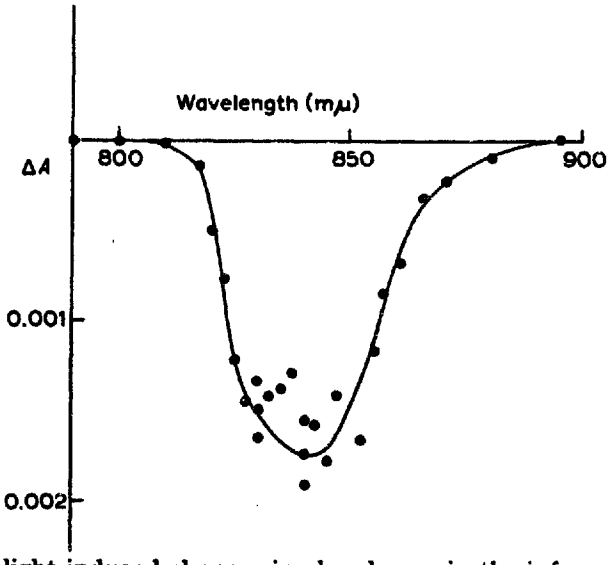

Fig. 1. Spectrum of the light-induced changes in absorbance in the infrared region for whole cells of *Cps. ethylicum*. The cells were taken from a 3-days-old culture and were resuspended in water.

When certain assumptions are made, a linear relation between the inverse fluorescence yield $1/\varphi$ of B 810 and the absorbancy change $\Delta A_{840\,m\mu}$ can be derived[1]. In Fig. 2 $1/\varphi$ is plotted as a function of ΔA. The graph appears to be linear in a first approximation.

The present results show that energy is transferred from B 810 to P 840 in a way similar to the energy transfer from bacteriochlorophyll B 890 to P 890 in *R. rubrum*[1].

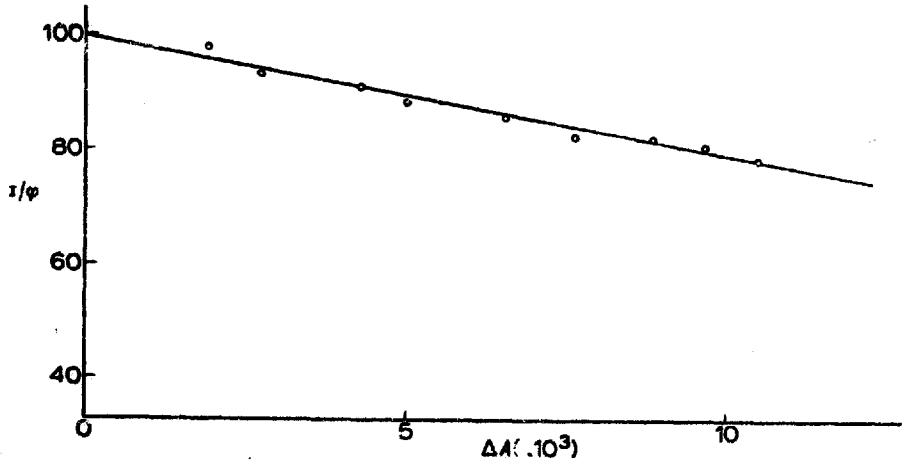

Fig. 2. The inverse fluorescence yield $1/\varphi$ plotted as a function of the change in absorbancy ΔA for cells suspended in water. The cells were taken from a 1-day-old culture of *Cps. ethylicum*.

The observed initial rate of bleaching of P 840 in non-inhibited cells and the rate of its regeneration in the dark indicate that regeneration of P 840 occurs immediately after the light-induced bleaching has started. In the PMA-treated cells this immediate regeneration is severely inhibited. The oxidation of cytochrome, which is clearly observed in the non-inhibited cells, could not be demonstrated in cells treated with $2 \cdot 10^{-5}$ M PMA. When a smaller concentration of PMA (10^{-5} M) is added a light-induced oxidation of cytochrome still could be observed but its reduction in the dark is slow and only partial. This indicates that in cells treated with $2 \cdot 10^{-5}$ M PMA the cytochrome is oxidized in the weak measuring light and remains oxidized in the course of the experiment. A comparison of the light-induced bleaching of P 840 with the light-induced oxidation of cytochrome in both non-inhibited and PMA-treated cells suggests that the inhibition of the electron transport at the site of the cytochrome leads to inhibition of the regeneration of P 840 in the dark. This can be seen as an argument for the assumption that the oxidation of cytochrome occurs through P 840 and that the light-induced bleaching of P 840 is a photo-oxidation. A difficulty, however, is the observation that under the present conditions and even at actinic light intensities at which the bleaching of P 840 is saturated, the increase of the fluorescence yield of B 810, although correlated with the P 840 bleaching, does not exceed 30%. The increase in B 810 fluorescence is larger than 30% if the fluorescence at 838 mμ is partially due to a constant fluorescence, not due to B 810. If this constant fluorescence is assumed to be 50% and the experimental points given in Fig. 2 are corrected accordingly the points do not fit the theoretically derived linear relation any more. This suggests that no constant background fluorescence occurs and that the transfer efficiency from B 810 to P 840 is low. Another possibility is that P 840 is bleached only partly and that the energy transfer from B 810 to "bleached" P 840 is still appreciable.

Further experiments need to be done in order to establish the nature and function of P 840 with more certainty.

We wish to thank Dr. J. M. OLSON, Brookhaven National Laboratory, Upton, New York, for giving us a culture of *Cps. ethylicum*, and Dr. L. N. M. DUYSENS for fruitful discussion.

Biophysical Laboratory, State University, Leiden (The Netherlands)

CHR. SYBESMA
W. J. VREDENBERG

1 W. J. VREDENBERG AND L. N. M. DUYSENS, *Nature*, 197 (1963) 355.
2 L. N. M. DUYSENS, W. J. HUISKAMP, J. J. VOS AND J. M. VAN DER HART, *Biochim. Biophys. Acta*, 19 (1956) 188.
3 R. K. CLAYTON, *J. Photochem. Photobiol.*, 1 (1962) 201.
4 J. M. OLSON AND C. A. ROMANO, *Biochim. Biophys. Acta*, 59 (1962) 726.
5 J. M. OLSON, D. FILMER, R. RADLOFF, C. A. ROMANO AND C. SYBESMA, *Symp. Bacterial Photosynthesis, Yellow Springs, Ohio, 1963*, in the press.
6 L. N. M. DUYSENS, *Thesis*, State University Utrecht, 1952.
7 C. SYBESMA AND J. M. OLSON, *Proc. Natl. Acad. Sci. U.S.*, 49 (1963) 248.
8 J. M. OLSON AND C. SYBESMA, *Symp. Bacterial Photosynthesis, Yellow Springs, Ohio, 1963*, in the press.
9 E. N. KONDRAT'EVA AND L. V. MOSHENTSEVA, *Doklady Akad. Nauk. SSSR*, 135 (1960) 460.
10 L. N. M. DUYSENS, in *Progress in Photobiology, Proc. 3rd Intern. Congr. Photobiol., Copenhagen, 1960*, Elsevier, Amsterdam, 1961, p. 135.

Received July 22nd, 1963