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LIGHT-INDUCED ABSORBANCE CHANGES IN THE GREEN PHOTOSYNTHETIC BACTERIUM *CHLOROPSEUDOMONAS ETHYLICUM*

C. SYBESMA* AND T. BEUGELING
Biophysical Laboratory of the State University, Leyden (The Netherlands)
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SUMMARY

1. Quantum requirements for the light-induced bleaching of P 840 in the green photosynthetic bacterium *Chloropseudomonas ethylicum* were determined with actinic light mainly absorbed by chlorobium chlorophyll under a variety of conditions. The estimated values ranged from 12 to 8.

2. Light *minus* dark absorbance difference spectra of *Cps. ethylicum* suspended in distilled water indicate the participation of two cytochromes.

3. Light-induced absorbance changes in the green spectral region of *Cps. ethylicum* suspended in distilled water suggest the reversible light-induced formation of a carotenoid-like pigment. This pigment may be related to carotenoid synthesis.

INTRODUCTION

Experiments on the green photosynthetic bacteria have demonstrated the light-induced reactions of one or more cytochromes[1] and a pigment, P 840, with a maximum absorption at about 840 nm (refs. 2, 3). Some evidence has been presented[2,3] which suggested that in green bacteria P 840 may be the site of a primary reaction of photosynthesis: phenyl mercuric acetate, which probably inhibits the dark reduction of cytochrome, does not alter the rate of the photobleaching of P 840 but inhibits its regeneration in the dark. Similar effects were found with a lowering of temperature[3]; the dark reduction of cytochrome is inhibited and the rate of regeneration of bleached P 840 in the dark is inhibited.

Under conditions in which the rate of the dark regeneration of P 840 is low (low temperature, addition of phenyl mercuric acetate) the lowest estimated quantum requirement for P 840 bleaching was 10 (C. SYBESMA AND W. J. VREDENBERG, unpublished). Earlier experiments[1] led to a lowest quantum requirement for cytochrome oxidation of 2. In view of this difference, further studies were performed on reaction rates of P 840 bleaching and cytochrome oxidation in the green photosynthetic bacterium *Chloropseudomonas ethylicum* under conditions in which a low rate of dark reactions could be expected. Under such conditions the kinetics of absorbance changes in the blue spectral region suggested the involvement of a number of cytochrome

* Present address: University of Illinois, Dept. of Botany, Urbana, Ill, U.S.A. Reprints can be requested from the Leyden Laboratory Schelpenkade 14A, Leyden (The Netherlands).

pigments in the light-induced reactions. Therefore a thorough scan of the spectrum of the light-induced absorbance changes was also made.

MATERIALS AND METHODS

Cps. ethylicum strain 2K was grown anaerobically in the light in a medium containing 0.2 % ethanol[4,5] in closed bottles. Prior to the experiments, the organisms from a 1-day-old culture were spun down in a cooled centrifuge at 35000 × *g* and resuspended either in distilled water or in a high-viscosity medium containing 75 % (v/v) of a 50 % (w/w) solution of potassium glycerophosphate and 25 % (v/v) glycerol at pH 7.0. In some cases samples were taken straight from the culture. Care was taken to minimize mixing with air. The samples were measured in 1-mm-thick glass cuvettes.

The experiments were carried out with a split-beam differential spectrophotometer described previously[6]. Actinic illumination was obtained from an Aldis slide projector. The filters used for actinic illumination were a Schott 740-nm AL interference filter in combination with a Balzers Calfex B1K1 infrared filter for far-red illumination, and a combination of the Schott glass color filters BG 12, BG 18 and BG 38, each 2 mm thick, for blue illumination (maximum at 435 nm). The intensity of the actinic light in most experiments was about $0.2 \cdot 10^{-9}$ Einstein $\cdot cm^{-2} \cdot sec^{-1}$ for far-red illumination and about $0.8 \cdot 10^{-9}$ Einstein $\cdot cm^{-2} \cdot sec^{-1}$ for blue illumination.

The experiments at temperatures of near 0° were carried out by means of a sample holder designed by VREDENBERG[7] with ice–water as a coolant.

Absorption spectra were measured with a Unicam SP 700 recording spectrophotometer.

RESULTS

The quantum requirement for P 840 bleaching in *Cps. ethylicum* was determined for the cells suspended in distilled water or in a mixture of potassium glycerophosphate and glycerol and at temperatures from 20° to 0°. The extinction coefficient at 840 nm was assumed to be 10^5 $M^{-1} \cdot cm^{-1}$ which is approximately the extinction coefficient, in the far-red spectral region, for bacteriochlorophyll in purple bacteria[8]. The values of the quantum requirement for P 840 bleaching, estimated from a dozen experiments under a variety of conditions and within a range of intensities of far-red and blue illumination, ranged from 12 to 8.

Suspending the cells in a mixture of potassium glycerophosphate and glycerol led to a severe inhibition of the dark reduction at 20° of cytochrome (measured at 425 nm). This is demonstrated in Fig. 1, where light-induced absorbance changes at 840 nm are compared with light-induced absorbance changes at 425 nm in the same sample illuminated with the same intensity ($0.2 \cdot 10^{-9}$ Einstein $\cdot cm^{-2} \cdot sec^{-1}$) of far-red (740 nm) light. Although the dark regeneration of P 840 is also inhibited by the medium, regeneration seemed to be complete within a few seconds, which is certainly not the case with the dark reduction of the cytochrome. This phenomenon has been observed earlier with P 890 and cytochrome in purple bacteria[6,8] and with P 700 and cytochrome in green plants[7].

When cells of *Cps. ethylicum* suspended in distilled water were examined for light-induced absorbance changes, a very slow reversible 'increase in absorbance'

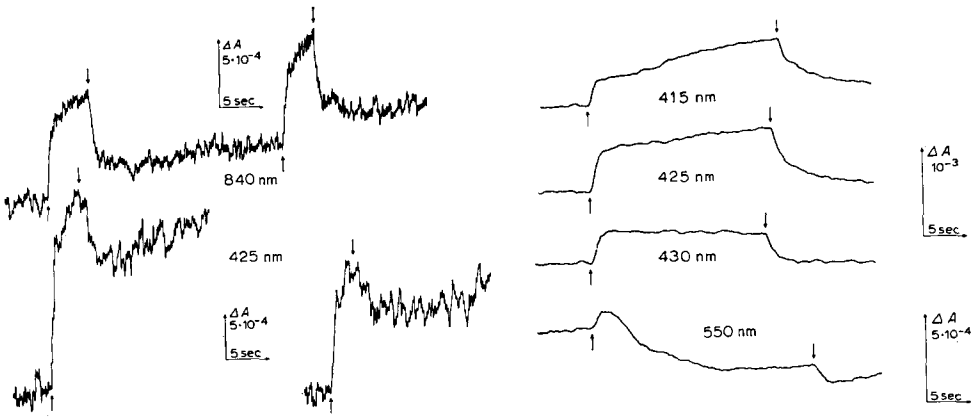

Fig. 1. A comparison of the kinetics of absorbance changes at 840 nm (upper tracings) and at 425 nm (lower tracings) of whole cells of a 1-day-old culture of *Cps. ethylicum* suspended in a medium containing 75% (v/v) of a 50% (w/w) solution of potassium glycerophosphate and 25% (v/v) glycerol. The switching on and off of the actinic light at 740 nm (intensity of $0.2 \cdot 10^{-9}$ Einstein$\cdot$cm$^{-2}\cdot$sec^{-1}) is indicated by upward and downward pointing arrows, respectively.

Fig. 2. The kinetics of absorbance changes at various wavelengths for whole cells from a 1-day-old culture of *Cps. ethylicum* suspended in distilled water. The switching on and off of the actinic light at 740 nm (intensity of $0.2 \cdot 10^{-9}$ Einstein$\cdot$cm$^{-2}\cdot$sec^{-1}) is indicated by upward and downward pointing arrows, respectively. The dark period between illuminations was 30 sec.

was observed in the wavelength region from 380 nm to 900 nm. This slow change was not very reproducible and is probably due to reactions not directly associated with photosynthesis. If, however, the dark period after each period of illumination was kept within about 30 sec, the kinetics of the absorbance changes were much more reproducible and show a multiphasic character. This was not (or to a much lesser extent) the case when the cells were suspended in growth medium, or at the very beginning of the experiment with depleted cells. Under such conditions only a fast change due to a cytochrome was observed. In Fig. 2 the kinetics of light-induced

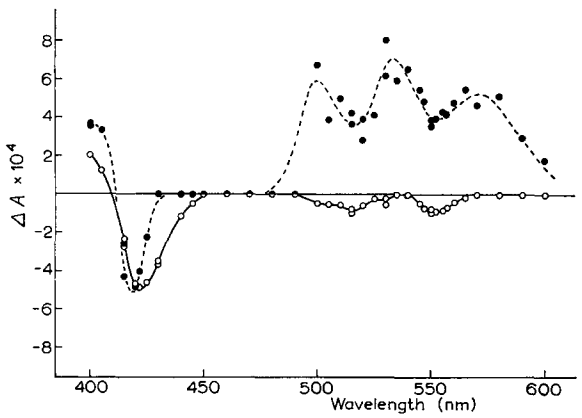

Fig. 3. Spectrum of light-induced absorbance changes in whole cells from a 1-day-old culture of *Cps. ethylicum* suspended in distilled water. Illumination periods of 20 sec were separated by dark periods of 30 sec. ○, fast initial phase; ●, total steady state *minus* fast initial phase.

absorbance changes at a number of wavelengths are shown for cells suspended in water and subjected to an illumination regime of 20 sec light followed by 30 sec darkness. It is clear that a fast initial phase is followed by a slower phase which reaches a steady state well within the illumination period and which has a different spectrum. A scan over the wavelength region from 380 to 600 nm resulted in a light *minus* dark absorbance difference spectrum, a typical example of which is shown in Fig. 3. The fast initial phase has a spectrum which is very similar to the cytochrome 422 spectrum observed previously[1]. The slower phase shows a peak in the Soret region which could be due to a second cytochrome with a band at about 419 nm.

In the green spectral region an increase in absorbance is observed with a rather complicated spectrum. The 3 peaks at about 500, 535 and 575 nm suggest the reversible light-induced synthesis of a carotenoid-like pigment, although no carotenoid with bands shifted to the red is yet known. No band shifts are found like the shifts observed by SMITH AND RAMIREZ[9] in purple bacteria. In order to check whether some kind of a light-induced synthesis was involved, absorption spectra of *Cps. ethylicum* of different ages were measured. Fig. 4 shows the absorption spectra of a 7-day-old culture and a 1-day-old culture of *Cps. ethylicum*. The main peaks at 745 nm are equalized. The aged cells show an increase in absorption in the region around 510 nm as compared with the young cells.

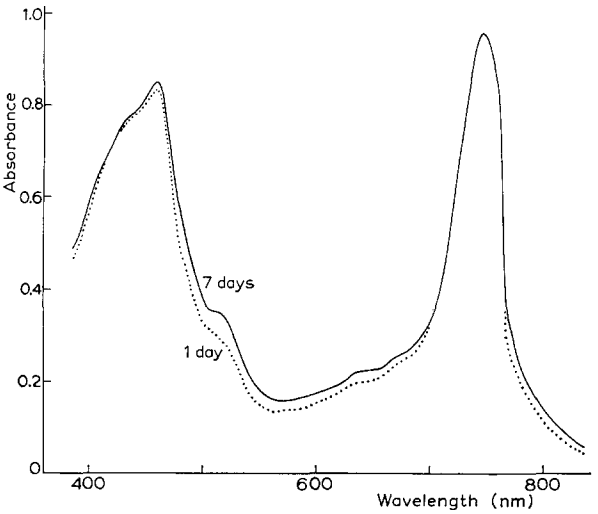

Fig. 4. Absorption spectra of a 7-day-old culture and a 1-day-old culture of *Cps. ethylicum* in growth medium. The absorption spectra were equalized at 745 nm.

DISCUSSION

The relatively large value of the quantum requirement for P 840 bleaching as compared with the quantum requirement for cytochrome oxidation[1] is difficult to explain, if the hypothesis that P 840 is a site of a primary reaction of photosynthesis is correct. However, the quantum requirement for cytochrome oxidation was measured with actinic illumination at 810 nm (ref. 1) which is near the maximum absorption of B 810, while in the present experiments P 840 bleaching was measured with actinic

illumination either at 435 or at 740 nm. If the energy transfer from B 810 to P 840 is very effective as compared with the energy transfer from chlorobium chlorophyll and carotenoid to B 810, the quantum requirement for P 840 bleaching will be lower when illumination at 810 nm is used. On the other hand, one would expect that the quantum requirement for cytochrome oxidation with actinic illumination at 740 nm would be higher. Indeed, values close to 5 were measured for the quantum requirement for cytochrome oxidation with actinic illumination at 740 nm (C. SYBESMA AND W. J. VREDENBERG, unpublished). The relatively high value of the quantum requirement for P 840 bleaching may be explained by the assumption made earlier[2] that P 840 is bleached only partially.

The light-induced absorbance changes in the blue spectral region measured with cells of *Cps. ethylicum* in distilled water (Fig. 3) suggest the participation of a second cytochrome, which we shall call cytochrome 419. Such a cytochrome was also found in the purple bacterium *Rhodospirillum rubrum*[10]. This cytochrome, which has a Soret peak (at 420 nm) shifted to the blue, is seen in this organism too when the cells are suspended in distilled water. There are indications that such a cytochrome also exists in *Chromatium*[7]. GIBSON[11] found two distinct cytochromes with spectra very close to each other in *Chlorobium thiosulphatophylum*, another species of green photosynthetic bacteria.

The slow changes in the green spectral region suggest a reversible light-induced formation of a carotenoid-like pigment.

If the absorption spectrum (equalized at 745 nm) of an aged culture is compared with the absorption spectrum of a young culture, more absorption is present in the carotenoid region of the old culture (see Fig. 4).

It is possible that the observed light-induced absorbance changes are related to the synthesis of carotenoids.

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