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EVIDENCE FOR A CYTOCHROME *b* IN GREEN BACTERIA

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Summary

Chemical and photochemical evidence is presented for a low potential cytochrome *b* (C563) bound to the photochemical reaction center complex of green bacteria. This cytochrome undergoes reversible light-induced oxidation. The midpoint potential lies between 0.0 V and −150 mV. It is present in about the same concentration as that of the particle-bound *c*-type cytochrome (C553) which was described earlier. There are four molecules of each cytochrome per P840 or 60—80 bacteriochlorophylls. Some similarities between the green bacterial photosystem and Photosystem I of green plants are discussed.

All known photosynthetic organisms with the exception of the obligate anaerobic purple bacteria and the green bacteria have been shown to contain "*b*"-type cytochromes. In *Chromatium*, Cusanovitch and Kamen [1] observed only *c*-type cytochromes, a low potential one associated with flavin, a *cc'* prime type and a particle-bound cytochrome C555. Meyer et al. [2] made an exhaustive study of the soluble cytochromes in the green bacterium *Chlorobium thiosulfatophilum* and found only *c*-type cytochromes. Fowler et al. [3] have isolated, from both *Chlorobium limicola* and *C. thiosulfatophilum*, a photochemically active reaction center complex. This complex is largely devoid of *Chlorobium* chlorophyll and retains all of the 60—80 bacteriochlorophyll *a* molecules per P840 found in the whole cell. It is photochemically active exhibiting a reversible light-induced bleaching of the reaction center P840 and photooxidation of a *c*-type cytochrome with an α band at 553 nm.

This paper presents evidence that the reaction center complex contains in addition a *b*-type cytochrome. It is therefore the first observation of such a cytochrome in these organisms.

Procedures for growing *C. limicola* and *C. thiosulfatophilum* and preparing the photochemically active complex were described previously [3, 4]. Light-induced absorbance changes were measured with an Aminco Chance dual wavelength spectrophotometer as described elsewhere [3]. Redox potential measurements were made with a Radiometer No. 126 pH meter and a Radiometer combination platinum electrode No. PK 149 in a special anaerobic cuvette arrangement [3]. Anthroquinone-1,5-disulfonate (E_{m7} = −170 mV) (10 μm) and 1,4-naphthoquinone (E_{m7} = +60 mV) (10 μm) were used as redox buffers during light-induced measurements.

The chemically induced oxidized minus reduced spectra presented in Fig. 1 clearly show that there are at least two bound cytochromes in the reaction center complex isolated from *C. limicola*. In Fig. 1a, which is the ferricyanide oxidized minus ascorbate reduced spectrum, we observe the disappearance of an α peak at 553 nm and a β peak at 522 nm. Not shown is the Soret minimum at 420 nm. The spectral and redox properties of this cytochrome were previously described [3]. The ascorbate reduced minus the dithionite reduced spectrum shown in Fig. 1c suggests that a single cytochrome having a redox potential below 0.0 V also is contained in the complex. The positions of the α peak (562 nm) and the β peak (533 nm) suggest strongly that this is a *b*-type cytochrome. In the completely oxidized minus reduced spectrum shown in Fig. 1b, both cytochromes are evident. Based upon reasonable extinction coefficients these cytochrome hemes are present in approximately equimolar amounts. There are approximately four of each type of cytochrome per 80 bacterio-

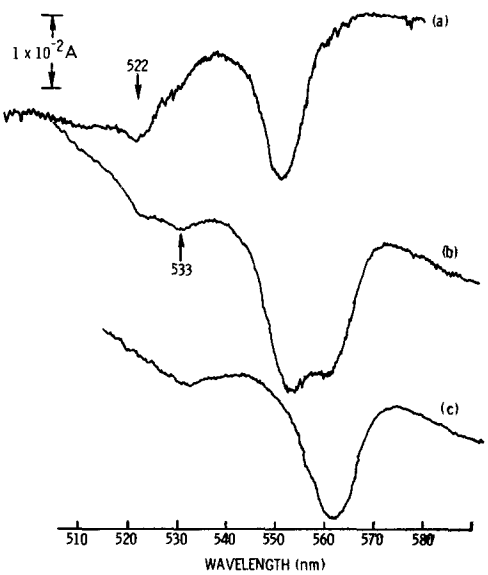

Fig. 1. Chemically induced oxidized minus reduced spectra measured in the α and β spectral region for the reaction center complex isolated from *C. limicola*. (a) Ferricyanide oxidized minus ascorbate reduced; (b) ferricyanide oxidized minus dithionite reduced; (c) ascorbate reduced minus dithionite reduced. Absorbance at 810 nm = 1.6.

chlorophyll *a* molecules or reaction center P840. No spectral evidence for a *b*-type cytochrome in the soluble fraction was obtained, which agrees with Meyer et al. [2].

It was subsequently found, but not shown here, that the reaction center complex isolated from *C. thiosulfatophilum* also contained this *b*-type cytochrome in approximately the same quantity.

In a previous paper we showed that the *c*-type cytochrome in the reaction center complex from both *C. limicola* and *C. thiosulfatophilum* undergoes a reversible light-induced oxidation [3]. In the redox range above 0.0 V only this cytochrome was observed to be oxidized in the light. Attempts to observe the reduction of cytochrome *b* in the light were negative. However, as the potential was lowered so that this cytochrome became reduced in the dark, a reversible light-induced oxidation could be measured. For example, when the redox potential was preset at −250 mV only the *b* cytochrome exhibited a reversible oxidation with peaks at 562, 530 and 428 nm, comparing well with the chemically oxidized minus reduced spectrum of this cytochrome shown in Fig. 1c. A complete spectrum is shown in Fig. 2. For comparison the light minus dark spectrum for the *c*-type cytochrome is also presented. The α-band is displayed on an expanded scale in Fig. 3.

When the external potential of the sample was raised, but still maintained above 0.0 V, both cytochromes were reversibly oxidized by illumination. For example, when the potential was preset at −150 mV (see Fig. 3), the α-peak became broader, with a minimum of 561 nm and a shoulder near 550 nm. When the spectrum obtained at −250 mV was subtracted from this spectrum (as is shown in Fig. 3), a symmetrical band centered at 554 nm was obtained.

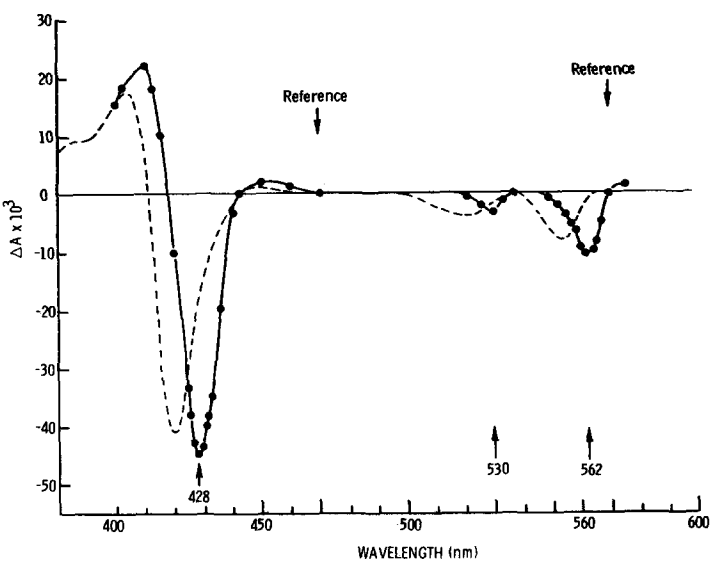

Fig. 2. Complete light minus dark spectra of the cytochromes in the reaction center complex from *C. limicola*. - - - -, sample redox potential set at 50 mV; ●——●, redox potential set at −250 mV.

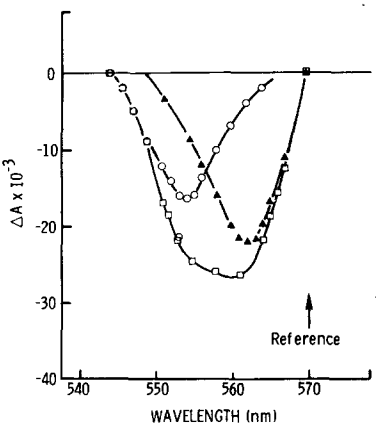

Fig. 3. Light minus dark spectrum in the α region for the reaction center complex from *C. limicola* preset at two ambient redox potentials. ▲——▲, −250 mV; □——□, −150 mV; ○——○, difference spectrum (□ minus ▲).

Under the conditions just described, where both cytochromes are oxidized in the light, differences in the dark recovery kinetics could also be observed. Examples of the light on and the light off kinetics are demonstrated in Fig. 4 at three selected wavelengths in a sample whose redox potential was set at −150 mV. The dark recovery switches from a predominantly fast recovery at 550 nm to a slower recovery at 564 nm. Definite biphasic kinetics occur at 558 nm indicating a mixture of the two decays: a plot of the fast and slow recovery phases (not shown) indicates that the fast recovery is associated with the *c*-type cytochrome and the slow recovery with the *b*-type cytochrome.

We again obtained almost identical results with the reaction center complex isolated from *C. thiosulfatophilum*.

The results show that there is a low potential cytochrome in green bacteria which can undergo reversible light-induced oxidation. Its spectral properties suggest that it is a *b*-type cytochrome. It is present only in the particulate fraction which contains the photochemical apparatus, and in a concentration, on a heme basis, equal to that of the higher potential *c*-type cytochrome. Thus there are approximately four *c*- and four *b*-type cytochrome hemes per reaction center P840, i.e. per 80 bacteriochlorophyll *a* molecules. The redox potential is below 0.0 V but certainly not lower than −150 mV. It might be of interest to point out that the peak positions and the potential of this cytochrome are very similar to the cytochrome *b* 563 which occurs in Photosystem I in green plants [5]. This adds still another similarity between the photosystem of the green bacteria and that of Photosystem I of green plants. For example, (1) the spectral properties of the bleaching of P840 in green bacteria closely resemble those of the bleaching of P700 in green plants [3, 6]; (2) the chlorophyll proteins isolated from the two types of organisms appear to be similar [7]; and (3) evidence has been presented that green bacteria can reduce NAD directly via ferredoxin [8]. Thus from an evolu-

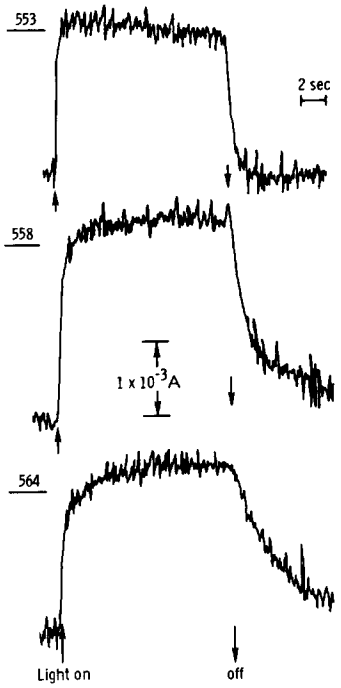

Fig. 4. Light on and light off kinetics for cytochrome oxidation and reduction at three selected wavelengths.

tionary viewpoint Photosystem I of green plants seems more closely related to the photosystem of green bacteria than to that of purple bacteria.

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