A REEVALUATION OF THE PRESENCE OF LOW MIDPOINT POTENTIAL CYTOCHROME 551.5 IN THE GREEN PHOTOSYNTHETIC BACTERIUM CHLOROPSEUDOMONAS ETHYLICA[1]

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SUMMARY

A soluble cytochrome with an α band having a maximum absorbance at 551.5 nm when reduced has been reported to be present in Chloropseudomonas ethylica. Recent results indicate that all C. ethylica strain 2-K cultures examined in this laboratory to date are mixtures of a photosynthetic bacterium, Chlorobium limicola, and at least one colorless heterotrophe. At least one type of colorless bacterium in C. ethylica cultures reduces oxidized forms of sulfur. It is proposed that the low midpoint redox potential, soluble c_3 cytochrome 551.5 found in cultures previously designated C. ethylica, is contained in such a sulfur reducer. The cytochrome is absent from the only photosynthetic microbe (Chlorobium) isolated from such mixtures.

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INTRODUCTION

A soluble cytochrome having absorption maxima at 418, 523 and 551.5 nm when reduced was reported to be present in the photosynthetic bacterium, C. ethylica (1). Subsequently, Meyer et al. isolated this cytochrome 551.5 from C. ethylica and characterized it extensively. They determined that the cytochrome was a c_3 type, low midpoint redox potential (Em, 7 = -194 m volts), compound found in appreciable concentration in C. ethylica (2,3). The amino acid sequence for C. ethylica cytochrome 551.5 has been elucidated by Ambler (4). The cytochrome has 68 amino acid residues, a molecular weight of 9100 and three haems per peptide chain (4). The only other photosynthetic bacteria reportedly containing low midpoint redox potential c_3 cytochromes are Rhodopseudomonas palustris and Rhodopseudomonas spheroides (2,3).

It has been known for several years that sulfate-reducing bacteria in the genus Desulfovibrio also have soluble low midpoint redox potential c_3 cytochromes (5,6). Moreover, amino acid sequences have been determined for some of these c_3 cytochromes isolated from different Desulfovibrio species (7,8,9). The amino acid sequences of c_3 cytochromes from Desulfovibrio species show striking homologies to the amino acid sequence of the low midpoint redox potential c_3 cytochrome 551.5 isolated from C. ethylica mixed cultures (4).

Recent results demonstrate that many C. ethylica cultures, including that culture from which cytochrome C551.5 was isolated, are mixtures of green, non-motile Chlorobium limicola and colorless bacteria capable of reducing oxidized forms of sulfur (10). C. ethylica cultures examined in our laboratory contain C. limicola and motile colorless heterotrophes. A full report of this syntrophism and its various implications is in the press (10). This communication presents evidence that cytochrome C551.5 is absent in pure cultures of Chlorobium limicola isolated from mixed cultures formerly called C. ethylica.

MATERIALS AND METHODS

Growth and Harvesting of Cells. C. ethylica was grown as previously described (11). Acetate was substituted for ethanol in the media. Chlorobium

limicola isolated from such cultures (10) was grown in the same media by increasing the sodium sulfide concentration ten fold to 0.2%. Elemental sulfur was removed from cultures by centrifugation at 10,000 x g for two minutes. Cells were harvested by centrifugation for ten minutes at 10,000 x g. They were washed once in 0.1 M potassium phosphate buffer (pH 7.5) containing 0.01 M sodium ascorbate.

Preparation of Cell-Free Extracts. 80% Methanol extracts were used to estimate Chlorobium chlorophyll (12). Cell suspensions were adjusted to 1.0 mg chlorophyll per ml, ten ml were passed through a French press at 18,000-20,000 psi and the slurry was centrifuged at 10,000 x g for 15 min at 0-4° C. Two ml aliquots were removed from the supernatant fluids and diluted for cytochrome analysis. The remaining supernatant fluids were centrifuged for two hr at

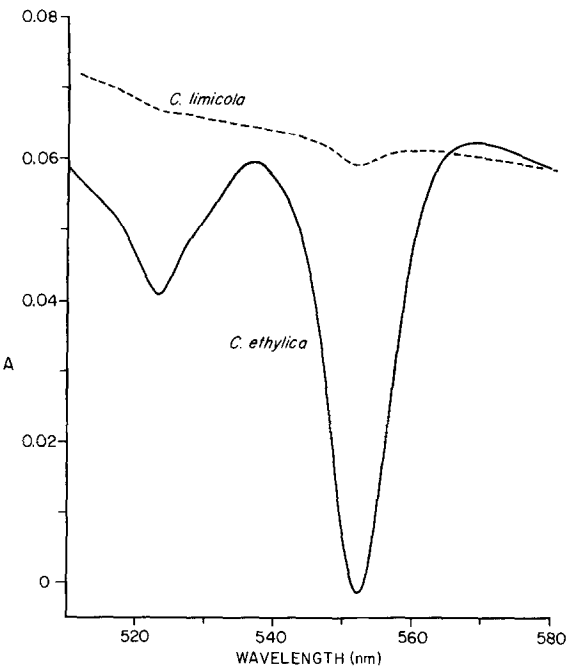

FIGURE 1. Oxidized minus reduced difference spectra of 150,000 x g supernatant fluids. C. limicola and C. ethylica 150,000 x g supernatant fluids were prepared as described in the Materials and Methods section. $Na_2S_2O_4$ was used to reduce the fluids and $K_3Fe(CN)_6$ was used to oxidize them. C. ethylica minima are at 522.7 and 551.5 nm. C. limicola has a minimum at about 553 nm.

40,000 rpm in a Beckman 50 titanium rotor (150,000 x g max). The supernatant fluids from this centrifugation were assayed directly for cytochromes.

Spectrophotometry. A Cary model 14 recording spectrophotometer equipped with an extended range photomultiplier type (RCA C31025C) was used to measure optical density (670 nm) and make absorption spectra. A sensitive slide wire (0.0-0.10D) was used to make difference spectra. $Na_2S_2O_4$ was the reductant and $K_3Fe(CN)_6$ was the oxidant in such measurements.

RESULTS AND DISCUSSION

Figure 1 shows the oxidized minus reduced difference spectra of 150,000 x g supernatant fluids obtained from cell-free extracts of cultures formerly designated C. ethylica and pure cultures of C. limicola isolated from the mixture. The prominent α band at 551.5 nm present in C. ethylica is absent in C. limicola. Only a modest 553.5 nm α band may be present in the C. limicola supernatant fluids.

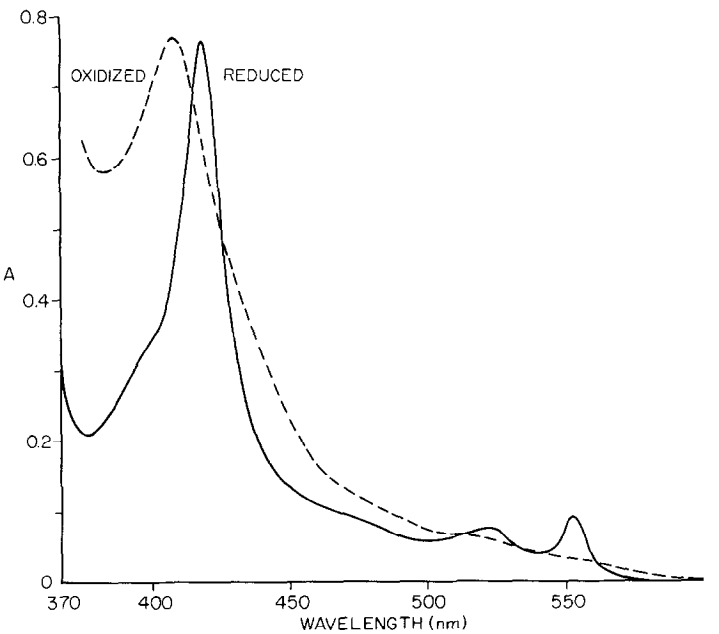

FIGURE 2. C. ethylica 150,000 x g supernatant fluids absorption spectra. C. ethylica 150,000 x g supernatant fluids were prepared as described in the Materials and Methods section. Fluid samples were oxidized with $K_3Fe(CN)_6$. Absorption spectra of such oxidized fluids have a maximum at 418 nm. Fluid samples were reduced with $Na_2S_2O_4$. Absorption spectra of such reduced fluids have maxima at 408, 523 and 552 nm.

Figure 2 is the oxidized and reduced spectra of 150,000 x g supernatant fluids obtained from cell-free extracts of C. ethylica. Although other proteins are undoubtedly present in such supernatant fluids, these spectra are similar to those made from highly purified preparations of cytochrome C551.5 (1,2,3).

Oxidized-minus-reduced difference spectra of 10,000 x g supernatant fluids and resuspended 10,000 x g pellets made from cell-free extracts obtained from pure cultures of C. limicola did not demonstrate the cytochrome 551.5 There was a modest minimum at about 553 nm in the 10,000 x g supernatant fluids made from cell-free extracts of C. ethylica.

Apparently mixed cultures designated C. ethylica contain cytochrome 551.5. This low potential c_3 cytochrome is probably present in sulfate-reducing bacteria contained in the mixture (10). It is important to note that the c_3 cytochrome present in C. ethylica cultures has three haems rather than four haems normally found in c_3 cytochromes obtained from most Desulfovibrio species (5). The possibility also exists that a sulfate-reducing bacterium present in C. ethylica cultures is an as yet undescribed species. Attempts are being made to isolate, in pure culture, a sulfate-reducing microbe from the mixture. C. limicola, the only photosynthetic bacterium isolated from C. ethylica mixtures, lacks cytochrome 551.5.

There is no widely accepted explanation for the presence of low midpoint redox potential c_3 cytochromes in photosynthetic bacteria. Meyer et al. were at a total loss when trying to explain the function of these c_3 cytochromes (2,3). The explanation may be simply that c_3 cytochromes are absent in pure cultures of Chlorobiaceae. The presence of low midpoint redox potential c_3 cytochromes in other photosynthetic bacteria may be similarly explained in the future.

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