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The low-potential cytochrome *c* of cyanobacteria and algae

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A water-soluble, low-potential cytochrome *c*-550 is found in some cyanobacteria and eukaryotic algae and has regions of sequence similarity to cytochrome c_6. This cytochrome appears to be involved in a fermentation that sustains the organisms during prolonged periods of dark, anaerobic conditions.

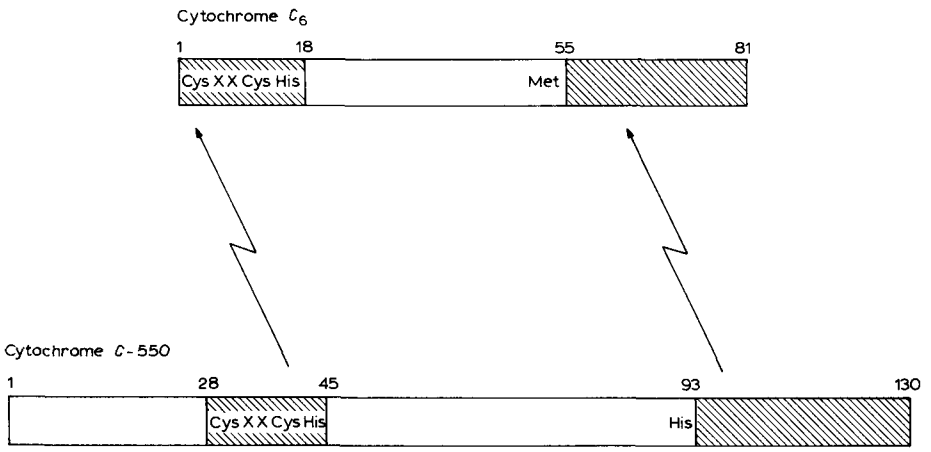

Fig. 1. Relationship between sequences of cytochromes *c*-550 and c_6.

A low-potential cytochrome *c*-550 (−260 mV) has been found in cyanobacteria [1,2], red and green algae [3,4] and a diatom [5]. The cytochrome contains a single heme and has a molecular mass of 15 kDa. The low redox potential resembles that of cytochromes c_3 of *Desulfovibrio,* but there is no similarity in the amino acid sequences. We first encountered this cytochrome in the fractionation of large-scale preparations of soluble proteins from cyanobacteria collected from natural blooms. In these natural blooms, the buoyancy of gas vesicles crowds the cells to the surface, forming a dense layer in which most of the cells have neither light nor oxygen. These blooms provide generous quantities of cells and we were able to determine amino acid sequences of several proteins from them.

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A bloom of *Microcystis aeruginosa* that occurred on the Potomac River in 1983 allowed us to complete the sequence of the low-potential cytochrome *c*-550 as well as the sequence of the better known cytochrome c_6 (E_m = +330 mV), which is an electron donor to Photosystem I in these organisms. The high-potential cytochrome has a molecular mass of 10 kDa and the sixth iron ligand is a methionine, as one would expect. Spectroscopic evidence indicates that the sixth ligand in the low-potential cytochrome *c*-550 is a histidine. Dr. Terry Meyer of the University of Arizona examined the sequences of the two cytochromes and recognized two regions of sequence similarity in the two proteins [6]. The cytochrome *c*-550 sequence from residue 28 to residue 45 is similar to the sequence of residue 1 to residue 18 in cytochrome c_6. This includes an alignment of the heme binding sites of *c*-550 of residues 37 and 40 with c_6 at residues 10 and 13. Another region of similarity is at the carboxy terminus of the two proteins. This

suggests that the gene for one of the proteins provided segments for the assembly of a gene for the second cytochrome. Fig. 1 illustrates this relationship.

The occurrence of the low-potential cytochrome in extracts of cyanbacteria is problematic. We have not found the cytochrome in laboratory-grown *Anabaena viriabilis*, but easily found it in laboratory-grown *Anacystis nidulans.* The cytochrome is found in extracts of laboratory-grown *Microcystis aeruginosa* and in all the samples of natural blooms. The cytochrome was absent from cells of *Spirulina maxima* growing optimally in commercial culture, but was abundant in cells from this culture after a processing shut down.

The cytochrome referred to here is, in all instances, a soluble protein. A membrane bound cytochrome which is similar in size, spectrum and redox properties has been observed in cyanobacteria. Krinner et al. [7] used Triton X-100 to release a cytochrome of molecular mass 15 000 from photosynthetic membranes of *Anabaena variabilis* that had previously been washed with cholate and octylglucoside. Bowes et al. [8] noted such a cytochrome in Photosystem II particles prepared by lauryl maltoside treatment of membranes of *Phormidium laminosum.* Hoganson et al. [9,10] washed membranes of *Anacystis nidulans* exhausively at low ionic strength and then released a similar cytochrome with 1 M NaCl. More critical data, including amino-acid sequences, are needed to determine whether these membrane-bound cytochromes are the same as the readily water-soluble one described in this paper.

In the cells from natural blooms and commercial cultures, we notices that the low-potential cytochrome was accompanied by a ferredoxin or a flavodoxin which were not found in laboratory grown cells. In all iron-sufficient cyanobacteria grown in the laboratory under conditions for optimal photosynthesis, one finds ferredoxin I, the strongly acidic protein widely known as the catalyst for supplying NADPH for CO_2 fixation. In iron-sufficient cells of natural blooms or dense commercial cultures, a less acidic ferredoxin II appears. Iron deficiency results in cells lacking both ferredoxins but containing abundant flavodoxin. As yet we have not distinguished two isozymic forms of the flavoprotein.

As a first step toward learning the biological function of this cytochrome, we attempted an enzymatic reduction using NADPH and the spinach chloroplast enzyme ferredoxin : $NADP^+$ oxidoreductase (FNR). The autooxidizability of the cytochrome requires that its reduction be observed under anaerobic conditions. A very low rate of cytochrome reduction was observed. When spinach ferredoxin I or ferredoxin I from cyanobacteria were added to the reaction mixture, a modest increase in the rate of cytochrome reduction occurred. Ferredoxin II gave a 10-fold greater rate than ferredoxin I. This degree of specificity for a ferredoxin is unusual in studies of photosynthetic tissue in which *Clostridium* ferredoxin works nearly as well as higher plant ferredoxin I in the chloroplast photoreduction of NADP. A preparation of ferredoxin II from the cyanobacterium *Aphanizomenon flos-aquae* was resolved by hydrophobic interaction chromatography into three distinct isoenzymes, each of which catalyzed the transfer of electrons from reduced FNR to low-potential cytochrome *c*-550 at a different rate.

Hutson et al. [11] have suggested that ferredoxin II in cyanobacteria participates in the phosphoroclastic oxidation of pyruvate – an activity which they and others have demonstrated in extracts of cyanobacteria. Many cyanobacteria do not seem to produce ferredoxin II when grown in the laboratory under conditions optimal for photosynthesis. We have found large amounts of ferredoxin II in the dense natural blooms and commercial cultures where many cells experience prolonged dark, anaerobic conditions. The specificity for ferredoxin II in the reduction of cytochrome *c*-550 and the often coincident appearance of ferredoxin II and cytochrome *c*-550 in cells experiencing dark, anaerobic conditions suggests that these proteins may link carbohydrate breakdown to disposal of electrons in a fermentative pathway. The low potential of this cytochrome is similar to that of cytochrome c_3 of *Desulfovibrio desulfuricans* and so one is prompted to look for an analogy in function.

Akagi described the series of reactions that link pyruvate oxidation via ferredoxin to cytochrome c_3 and then to hydrogenase in *D. desulfuricans* [12]. The laboratory at Marseille has documented the ferredoxin–cytochrome interaction in elegant detail. The catalysts for such a pathway and the circumstances for its use are present in cyanobacteria and many algae. Gaffron and Rubin [13] first described the hydrogen producing activity of eukaryotic algae and a recent survey has confirmed the very wide distribution of this enzyme [14]. Hallenbeck and Benemann [15] pointed out the hydrogenase in the cyanobacterium *Anabaena cylindrica* does not use ferredoxin as an electron donor, so mediation by the low-potential cytochrome is an attractive possibility. We are seeking evidence that the reduced cytochrome *c*-550 can transfer electrons to a cyanobacteiral hydrogenase. Finally, there is important evidence from studies of natural blooms of *Microcystis aeruginosa* in South Africa. Zohary has described hyperscums of *M. aeruginosa* that accumulate at the Hartbeespoort Dam to a depth of one meter and cover an area of several hectares [16,17]. The cells are aphotic and anaerobic and persist in a viable condition without loss of photosynthetic CO_2 fixation activity for several months. The densely compacted cell mass contains traces of hydrogen gas and acetic acid, despite the presence of heterotrophic bacteria which consume these products [18]. J. van der Oost [19] has described the participation of hydrogenase in the fermentation of carbon reserves by

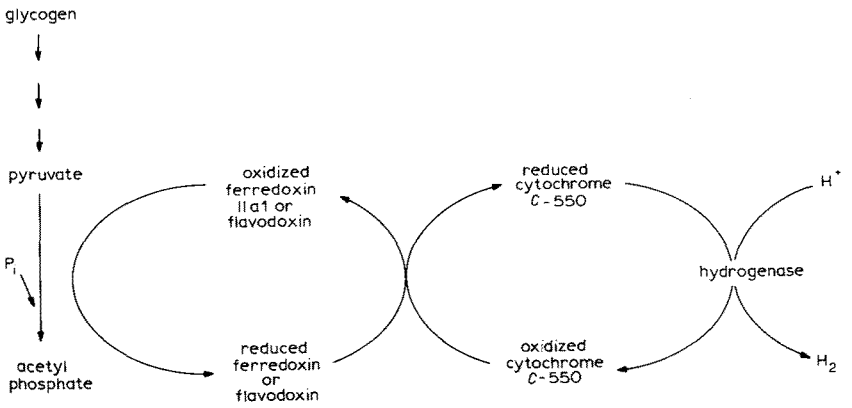

Fig. 2. Proposed pathway of anaerobic metabolism in *M. aeruginosa*.

Cyanothece in axenic cultures. The cells produce hydrogen, carbon dioxide, lactate, formate and acetate and were found to contain pyruvate-ferredoxin oxidoreductase, acetate kinase and reversible hydrogenase. Fig. 2 shows a pathway which includes the proposed reactions. Mahro et al. showed that *Chlorella fusca* with active hydrogenase maintained a higher level of ATP under dark, anaerobic conditions than did cells without this enzyme [20]. Kumazawa et al. correlated dark hydrogen evolution with maintenance of energy charge by unicellular green algae – *Chlorococcum* and *Chlamydomonas* during dark, anaerobic incubation [21]. Thus, a fermentation of carbohydrate reserves with protons and hydrogenase as the terminal oxidase for disposal of electrons as hydrogen gas could supply ATP for long-term maintenance of cells in the dark, anaerobic state.

While a few cyanobacteria and algae experience dark, anaerobic conditions as a result of bloom formation, this kind of experience from a different cause is general among small aquatic plants. In the temperate zone, many cyanobacteria and algae are known to sink to the bottom in autumn and become buried in silt. They pass the winter in a dark anaerobic condition and emerge to repopulate the water when the weather warms. This hibernation ability makes sensible the appearance of the low-potential cytochrome *c*-550 in the unicellular cyanobacteria and diatoms.

Perhaps this fermentation pathway has persisted in some higher plants which experience extended periods of anaerobiosis during germination in flooded soils. Torre et al. [22] have described an expression of hydrogenase activity in barley after anaerobic stress.

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