Isolation and characterization of the membrane-bound cytochrome \( c-554 \) from the thermophilic green photosynthetic bacterium \( Chloroflexus aurantiacus \)

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**Abstract**

The membrane-bound photooxidizable cytochrome \( c-554 \) from \( Chloroflexus aurantiacus \) has been purified. The purified protein runs as a single heme staining band on SDS-PAGE with an apparent molecular mass of 43 000 daltons. An extinction coefficient of \( 28 \pm 1 \) mM\(^{-1}\) cm\(^{-1}\) per heme at 554 nm was found for the dithionite-reduced protein. The potentiometric titration of the hemes takes place over an extended range, showing clearly that the protein does not contain a single heme in a well-defined site. The titration can be fit to a Nernst curve with midpoint potentials at 0, + 120, + 220 and + 300 mV vs the standard hydrogen electrode. Pyridine hemochrome analysis combined with a Lowry protein assay and the SDS-PAGE molecular weight indicates that there are a minimum of three, and probably four hemes per peptide. Amino acid analysis shows 5 histidine residues and 29% hydrophobic residues in the protein. This cytochrome appears to be functionally similar to the bound cytochrome from \( Rhodopseudomonas viridis \). Both cytochrome \( c-554 \) from \( C. aurantiacus \) and the four-heme cytochrome \( c-558-553 \) from \( R. viridis \) appear to act as direct electron donors to the special bacteriochlorophyll pair of the photosynthetic reaction center. They have a similar content of hydrophobic amino acids, but differ in isoelectric point, thermodynamic characteristics, spectral properties, and in their ability to be photooxidized at low temperature.

**Abbreviations:** LDAO – lauryl dimethyl amine-N-oxide, SDS-PAGE – sodium dodecyl sulfate polyacrylamide gel electrophoresis, mV – millivolt, \( E_{mK} \) – midpoint potential at pH 8.0, ODV – optical density × volume in ml

**Introduction**

The central involvement of cytochromes in electron transport processes has long been recognized. They operate in a variety of systems including the respiratory systems of mitochondria and bacteria, and in the photosynthetic apparatus of plants, algae, cyanobacteria, and the anoxygenic bacteria. They are involved as water soluble components, such as mitochondrial cytochrome \( c \), and bacterial cytochrome \( c_2 \), or as membrane-bound components such as the cytochrome \( b/c_1 \) complexes and cytochrome oxidase.

The membrane-bound cytochromes can be further separated into those which reside primarily in the membrane, such as cytochrome oxidase (Petti-grew and Moore 1987) and the cytochrome \( b \) portion of the cytochrome \( b/c_1 \) or \( b_6/f \) complexes (Widger et al. 1984), and those that have a hydrophobic anchor in the membrane and function primarily in
the adjacent aqueous environment. Examples of the latter class are cytochrome \( c_1 \), cytochrome \( f \) (Wakabayashi et al. 1982, Willey et al. 1984, Pettigrew and Moore 1987), and cytochrome \( c-558-553 \) from \textit{Rhodopseudomonas viridis} (Weyer et al. 1987a).

The membrane-bound multiheme cytochrome \( c-554 \) from the thermophilic green photosynthetic bacterium \textit{Chloroflexus aurantiacus} serves as the electron donor to the reaction center (Bruce et al. 1982, Blankenship et al. 1983, Blankenship 1985, Wynn et al. 1987, Zannoni and Venturoli 1988). This cytochrome is present only in photosynthetically grown cells, and follows an induction pattern similar to that of bacteriochlorophylls \( a \) and \( c \) and the reaction center upon shifting from aerobic to photosynthetic growth conditions (Pierson 1985, Foster et al. 1986).

\textit{C. aurantiacus} appears to have diverged from other photosynthetic organisms at a very early point (Woese 1987) and thus has a unique evolutionary history. It contains a reaction center similar to that of purple photosynthetic bacteria (Pierson and Castenholtz 1974, Bruce et al. 1982, Pierson and Thornber 1983, Blankenship 1985, Amesz 1987, Kirmaier and Holten 1987, Blankenship et al. 1988b, Ovchinnikov et al. 1988a, b), but an antenna system similar to the green sulfur bacteria (Olson 1980, Fieck and Fuller 1984, Blankenship et al. 1988a). \textit{C. aurantiacus} also appears to lack detectable water-soluble cytochromes, which are normally involved in bacterial photosynthesis (Bartsch 1978, Wynn et al. 1987). Instead, a blue copper protein may serve as a mobile electron carrier (Trost et al. 1988, McManus et al. 1988). The organism contains a very high heme \( c \) to protoheme ratio (Wynn et al. 1987). It has also been reported to have antimycin A and myxothiazol cytochrome \( b/c_1 \) inhibition patterns similar to the green plant cytochrome \( b_0/f \) complex (Zannoni 1987). This latter finding suggests that this complex is significantly different from the bacterial cytochrome \( b/c_1 \) complexes studied so far. This places \textit{C. aurantiacus} in a position that could contribute greatly to our understanding of the evolution of cytochromes in photosynthesis. Cytochrome \( c-554 \) has been purified and partially characterized in an effort to better understand photosynthetic electron transport in this unusual organism.

### Methods

#### Sources

All chemicals used were reagent grade unless specified otherwise. Ultrapure grade SDS was obtained from Boehringer Mannheim, Indianapolis, Indiana. Acrylamide and bis acrylamide were from Bio-Rad, Richmond, California. Pyocyanin was prepared as described by Prince et al. (1981).

#### Cell growth

\textit{C. aurantiacus} strain J10-fl was grown under high light conditions in the modified medium D of Pierson and Castenholtz (1974) at 55°C in a 16 liter fermenter. Cultures were routinely checked for purity by light microscopy. Cells were harvested by centrifugation at 12,000 \( \times \) \( g \) for 10 min.

#### Purification

All steps were carried out at 4°C unless stated otherwise. Wet-packed cell paste (150–200 g), was suspended in a total volume of 300 ml of 50 mM Tris pH 8.0 buffer containing 1 mM phenylmethylsufonyl fluoride as a protease inhibitor, cooled to 0°C, then disrupted with a Branson model 350 sonifier at a power setting of 8. Centrifugation at 12,000 \( \times \) \( g \) for 10 min removed unbroken cells and cellular debris. The membrane fragments were pelletted by centrifugation at 200,000 \( \times \) \( g \) for two hours in a Beckman 50.2 Ti rotor. In some instances the membranes were salt-washed to remove the blue copper protein auracyanin (Trost et al. 1988) and then repelleted as above. The pellets were suspended and adjusted to a final optical density of 16 at 865 nm in 50 mM Tris pH 8.0, 1.5% lauryl dimethyl amine-N-oxide (LDAO), and incubated with gentle shaking at 37°C for 1 h. After centrifugation at 200,000 \( \times \) \( g \) for two hours, the supernatant liquid, containing solubilized cytochrome, reaction centers, and free pigments, was dialyzed against 10 volumes of 50 mM Tris pH 9.0 for two days with a buffer change at 24 h. The dialyzed material was loaded on DEAE Sephalan anion exchange media (Phamacia) in a radial flow column.