The sequences proposed in Fig. 1 for the *Ps. aeruginosa* and *Ps. stutzeri* proteins have been confirmed by the isolation and characterization of the expected peptides from tryptic digests of the haem-free proteins. From each of these two proteins a second tryptic peptide was isolated that contained two residues of cysteine and a residue of histidine. The amino acid composition was such that the peptide could not have been derived from the region shown in Fig. 1.

The N-terminal heptapeptide sequence of the cytochromes *c*₄ is very similar to the N-terminus of *Rhodopseudomonas rubrum* cytochrome *c*₂ (Dus *et al.*, 1968). A similar N-terminal sequence, Tyr-Asp-Ala-Ala-Ala-Gly-Lys-, has also been detected in another monohaem cytochrome *c* from a photosynthetic bacterium, the cytochrome *c*-555 of *Chlorobium thiosulfatophilum* (Gibson, 1961; R. P. Ambler & T. Meyer, unpublished work).

The very close similarity between the cytochromes *c*₄ of *A. vinelandii* and the pseudomonads was unexpected. The organisms are very different in metabolism and in subcellular structure, and in the current classification (Breed *et al.*, 1957) are placed in different orders. However, they all have G+C contents of their DNA in the range 56–66% (Hill, 1966).

The dehalogenating pseudomonads contain a wide range of haem-*c*-containing proteins. In addition to cytochromes *c*-551, *c*₄ and *c*₅ they contain cytochrome *c* peroxidase (Kodama *&* Mori, 1969; Ellfolk & Soininen, 1970) and cytochrome *c* oxidase (Horio *et al.*, 1961). The interrelation of these components is still obscure (Horio & Kamen, 1970).

We thank Dr. T. Kodama for the gift of *Ps. stutzeri* cytochrome *c*₄ [*cytochrome c*-552 (II)] and Dr. T. Meyer for the *Rsp. rubrum* cytochrome *c*₂. The sequencer was purchased by the Medical Research Council, who support this part of the work.

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**The Amino Acid Sequence of *Chlorella fusca* Plastocyanin**

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Plastocyanin is a blue copper protein of low molecular weight. It was first found by Katoh (1960) in *Chlorella ellipsoidea*, and has since been shown to be present in the chloroplasts of green algae and higher plants, where it functions in photosynthetic electron transport (see, e.g., Arnon *et al.*, 1970).

For the present study the protein was isolated from spray-dried cells of *Chlorella fusca*, by a development of the method of Gorman & Levine (1966), in yields of about 0.7 mg of pure protein/g of dry cells. The amino acid sequence of the protein was investigated by
isolation and characterization of the peptides obtained from chymotrypsin and thermolysin digests of the protein. Since the protein contains no arginine and little lysine (4 residues) and has many bonds sensitive to cleavage by pseudotrypsin (Keil-Dlouha et al., 1971), tryptic digestion has proved to be of little use. The N-terminal sequence of the molecule was checked by using an automatic Sequenator (Beckman model 890; Edman & Begg, 1967); results were obtained as far as residue 36, and were in complete agreement with the sequence deduced by conventional methods. The structure deduced was a single polypeptide chain of 98 residues (Fig. 1).

Preliminary analyses indicate the presence of small amounts of carbohydrate, and electrophoretic mobilities suggest that a labile group may be associated with residue 9 or 10.

Incomplete experiments with plastocyanin from spinach (Brassica oleracea), Chlorella pyrenoidosa and Scenedesmus obliquus have shown very considerable similarities in sequence. The sequence of the plastocyanin from French bean (Phaseolus vulgaris) is currently under investigation by Milne & Wells (1970).

Katoh et al. (1961) suggested that the thiol groups of plastocyanin contributed to the binding of the copper. The proposed sequence contains only a single cysteine residue (position 83), clustered around by several aromatic residues. In the bacterial azurins, which are copper proteins that are believed to be of structure and function comparable with those of plastocyanin (Malkin & Malmstrom, 1970), there is a similar concentration of aromatic residues in the vicinity of the single thiol group (Fig. 2) (Ambler & Brown, 1967; Ambler, 1971). The sequence similarities are not sufficient to provide convincing evidence for homology between the two classes of proteins, but are striking enough to suggest at least a functional similarity. In both cases the residues concerned are close to the C-terminus of the molecule. Further studies [e.g. of the plastocyanin reported to be present in the blue-green alga Anabaena variabilis (Lightbody & Krogman, 1967)] may clarify the relationship.

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Amino Acid Sequence of Cytochrome c₅ from *Pseudomonas mendocina*

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Many bacteria contain several soluble c-type cytochromes. Both pseudomonads (Horio, 1958) and *Azotobacter* (cytochrome c₅; Tissieres, 1956) contain proteins with α-band maxima at about 555 nm. In studies of other cytochromes from pseudomonads, we have frequently met with cytochromes of the c₅ type, but chromatographic properties and yields have been very erratic. All purifications used have involved exposure to low pH values (pH 4.5; Ambler, 1963a), and Swank & Burris (1969) have shown instability of *Azotobacter* cytochrome c₅ at acid pH values. However, it has been found possible to isolate an adequately homogeneous cytochrome of this type from *Pseudomonas mendocina* CH-110 (Palleroni et al., 1970), in good yield.

The growth of the organism and the general procedures for the isolation of the protein were similar to those that have been described for *Pseudomonas* cytochrome c-551 (Ambler, 1963a). The major cytochrome present (c-551) was eluted from CM-cellulose at pH 4.45, and a mixture of the cytochrome c₅ and cytochrome c₄ (Ambler & Murray, 1973) was eluted at pH 4.75. The latter components were then separated by chromatography on DEAE-cellulose (at pH 8.5 in 12 mM-tris-HCl, with an NaCl gradient; cytochrome c₅ was eluted at about 32 mM-NaCl, ahead of cytochrome c₄), and further purified by (NH₄)₂SO₄ precipitation (70-95% saturation) and gel filtration through Sephadex G-75 (superfine grade; in 50 mM-ammonium acetate, pH 5.1). The yield of cytochrome c₅ was about 2.2 μmol/100 g of acetone-dried cells (compared with about 8 μmol of cytochrome c-551 and 0.12 μmol of cytochrome c₄).

This cytochrome c₅ was homogeneous by gel electrophoresis, and had a haem content (from visible spectrum) of about 1 residue/10000 daltons of amino acids. The behaviour of the protein on Sephadex G-75 was indistinguishable from that of the 9000-dalton cytochrome c-551. Amino acid analysis showed the complete absence of tyrosine and phenylalanine (<0.03 residue/hæm group), and a valine content of 1.1 residue/hæm group. Nevertheless N-terminal group analysis by the dansyl method showed both alanine and serine.

After removal of the haem (Hg₂Cl₂-0.1 mM-HCl-8 mM-urea, 37°C, 24 h), the apoprotein was digested with trypsin or chymotrypsin, and the peptides formed were isolated and characterized by standard methods (Ambler & Brown, 1967; Gray, 1972). The peptides could all be fitted together to form the unique sequence shown in Fig. 1, which agrees very well with the amino acid analysis results for the whole protein, and with the results of C-terminal analysis with carboxypeptidase A. However, at least four different tryptic peptides were derived from the N-terminal region of the molecule, and all the available...