

THE STRUCTURE OF CYTOCHROME c'_3 FROM DESULFOVIBRIO GIGAS (NCIB 9332)

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The species of the genus *Desulfovibrio* [1,2] differ markedly in their morphology and in their DNA base ratio [3,4], but have a generally similar metabolism, and all possess two diagnostic pigments, desulphoviridin and cytochrome c_3 . In this paper the amino acid sequences of cytochrome c_3 from two species of *Desulfovibrio* are compared.

In 1958 Postgate [5] isolated cytochrome c_3 from the organism now known as *D. vulgaris* (NCIB 8303; strain Hildenborough), and showed it to be similar to mammalian cytochrome c in molecular weight and absorption spectrum, but to have a remarkably low oxidation-reduction potential (-205 mV) and at least two haem groups per molecule. The amino acid sequence has been determined [6] and shows no similarity to that of other c -type cytochromes. In 1963 Le Gall [7] isolated a new species, *D. gigas* (NCIB 9332), and from it cytochrome c'_3 has been isolated [8]. This protein is similar to the original cytochrome c_3 in molecular weight and oxidation-reduction potential, but has a very different isoelectric point (pH 5.2 rather than 10.5), major differences in amino acid composition, and different terminal groups [9]. We have now examined the amino acid sequence of the protein (fig. 1), and are able to show that the sequence is so similar to that of cytochrome c_3 that the proteins must be considered homologous.

Cytochrome c'_3 was prepared as previously described [8]. The haem was removed by reduction

with HgCl_2 in 0.1 N HCl/8 M urea (25 mg/ml protein, 50 mg/ml HgCl_2 , 37° , 12 hr), and the apoprotein isolated by gel filtration through Sephadex G-25 into 5% (v/v) HCOOH . After performic acid oxidation the protein (40–50 mg) was digested with trypsin, chymotrypsin or thermolysin, and the peptides fractionated and their amino acid sequences investigated by standard methods [10,11]. The peptides characterized, and the amino acid sequence deduced are shown in fig. 1. It has not yet been possible to isolate a peptide linking residues 20 and 24. The tryptic peptide covering this region appears to have peculiar solubility properties, and several bonds in the region are susceptible to both chymotrypsin and thermolysin hydrolysis. It is postulated that the residue valine occurs at sites 21 and 22 and phenylalanine at site 23, as the amino acid analyses of the whole protein ([9], confirmed in this investigation) call for a residue of phenylalanine and probably two residues of valine in excess of those recovered in peptides, and these positions seem the only possible location for them consistent with the known specificity of the proteases used. A peptic peptide of probable composition (Val_2Phe) has been isolated but not completely characterized. There is an amide group on one only of the residues 34, 35, 39 or 42, but on which is not yet known. A chymotryptic peptide with properties consistent with it being Lys-Lys-Lys-Leu (residues 97–100) has been isolated, but has not been sufficiently characterized to include in the figure.

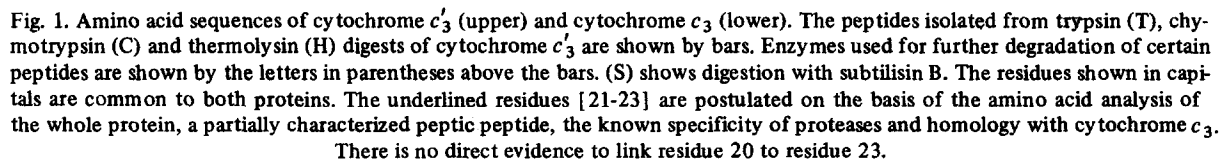


Fig. 1 also shows the sequence of cytochrome c_3 [6], aligned so as to give the best match with that of cytochrome c'_3 . The forty-nine residues in cytochrome c'_3 shown in capitals exactly match residues in cytochrome c_3 , if five deletions in cytochrome c_3 and one in cytochrome c'_3 are allowed. The four cysteine-histidine clusters, which must be assumed to be involved in haem binding, match exactly, though there is variation in the residues separating the cysteine pairs. The other histidine residues and the aromatic residues are mostly conserved, but the relatively few hydrophobic residues (valine, leucine, isoleucine and methionine) are not. Fifty residues are definitely not identically matched, but of these only twenty-three need be separated by a single mutational event.

Despite their great difference in morphology, *D. vulgaris* and *D. gigas* have similar DNA base ratios (60–61% G+C [2]). If this similarity is an indication that the species are more closely related to each other than to *D. desulfuricans* (55% G+C) or *D. saxilegens* (46% G+C) we may expect to find enormous differences in the amino acid sequences of the cytochromes c_3 of these latter organisms.

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