THE AMINO ACID SEQUENCE OF CYTOCHROME c_3 FROM *DESULFOVIBRIO DESULFURICANS* (STRAIN EL AGHEILA Z, NCIB 8380)

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In previous communications [1, 2] we have reported the amino acid sequences of the cytochromes c_3 from two species of the sulphate reducing bacterium *Desulfovibrio*. The different species in this genus [3] are similar in their general metabolism, but differ markedly in their DNA base composition [4, 5] and morphology. We have now determined the amino acid sequence of the cytochrome from a representative of a third species of the genus (table 1 and fig. 1). The sequence of the protein is sufficiently similar to that of the other two (fig. 2) for there to be no reasonable doubt that the three proteins are homologous.

The conditions for growth of the organism and preparation of the cytochrome were similar to those used for the preparation of *D. vulgaris* cytochrome c_3 [6]. The final preparation was judged to be pure by polyacrylamide and starch gel electrophoresis, and by *N*-terminal groups analysis, and by stoichiometry and absence of leucine in acid hydrolysates of the protein.

The apo-protein was prepared as previously described [2], and the amino acid sequence determined by separation and analysis by standard methods [7, 8] of the products of tryptic, chymotryptic and thermolysin digestion (fig. 1). Most of the details of the sequence were determined from a tryptic digest of 2.5 μmole performic-acid oxidised apo-protein, but the large peptide comprising residues 41–62 could not be obtained in good enough yield for complete sequence determination. The protein is completely lacking in leucine, and three of the five aromatic residues are very close together (residues 68–72), so chymotryptic digestion presented difficulties. By careful choice of conditions (pH 9, enzyme-substrate ratio 1/15 by wt, 4 hr, 37°), it was found possible to digest the unoxidized apo-protein such that cleavage occurred at most of the histidine and methionine residues. Even with very good quality chymotrypsin, pre-treated with soya-bean trypsin inhibitor, it was very easy to overdigest the protein to give a highly complex mix-

Table 1
Properties of cytochromes c_3 of known amino acid sequence, and of the bacteria from which they were prepared.

Organism	*D. vulgaris*	*D. gigas*	*D. desulfuricans*
	NCIB 8303	NCIB 9332	NCIB 8380
(strain)	Hildenborough		El Agheila Z
G + C%	61	60	55
Cytochrome c_3			
isoelectric point	10.5	5.2	about 10
No. of residues	106	110	102
Amino acids absent	Ile, Trp	Met, Arg	Leu

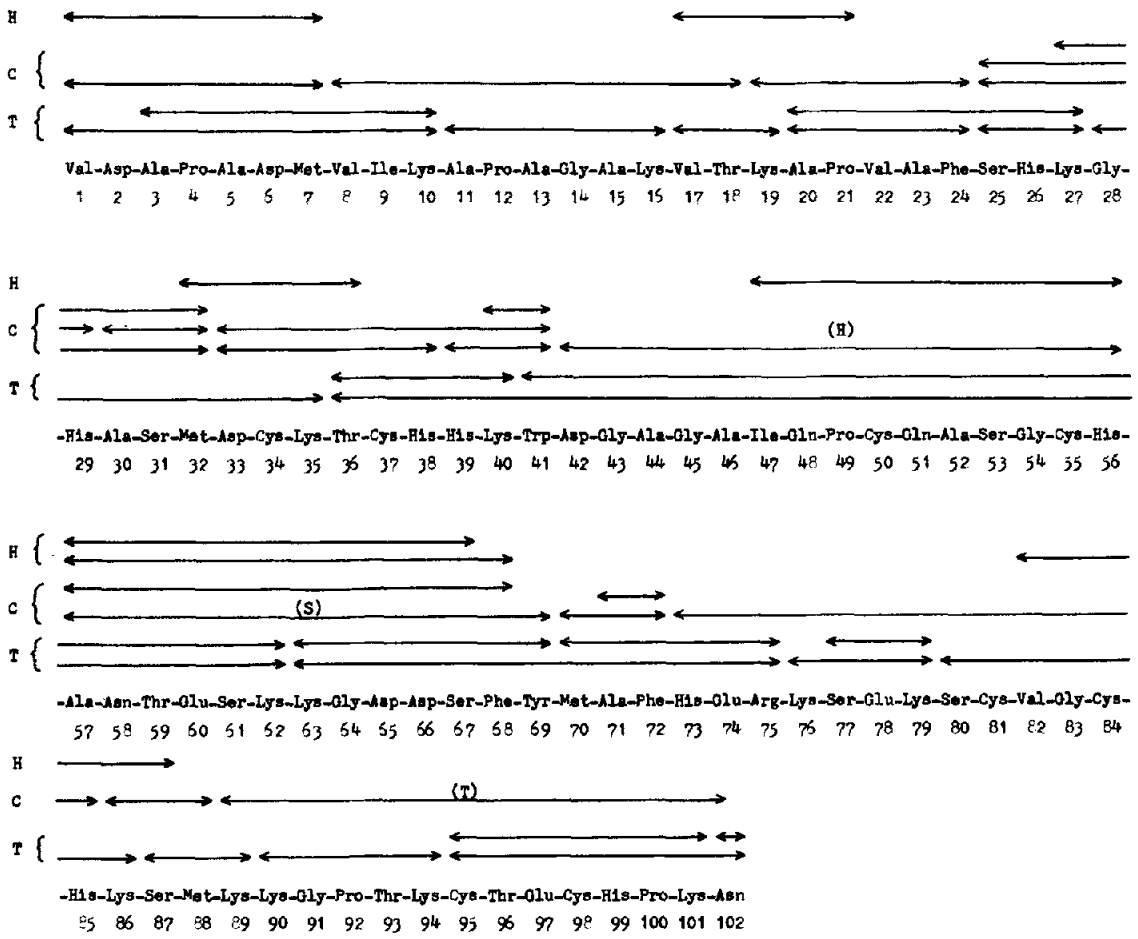

Fig. 1. Amino acid sequence of *D. desulfuricans* cytochrome c_3. The peptides isolated from trypsin (T), chymotrypsin (C) and thermolysin (H) digests are shown by bars. Enzymes used for further degradation of certain peptides are shown by the letters in parentheses above the bars. (S) shows digestion with subtilisin B. Where a digest contained two or more peptides derived from the same region of the cytochrome, the lower bar indicates the peptide recovered in highest yield.

ture in which many lysyl bonds had been hydrolysed. The Thr–Lys bond (18/19) was unexpectedly found to be very susceptible to chymotryptic hydrolysis. Difficulty was also experienced in estimating the tryptophan content of the protein. This cannot be done by direct spectrophotometry or by specific colour reactions when the haem is present, and it was found that the process of haem removal partially degraded the tryptophan. From the chymotryptic digests of the apo-protein, peptides His–Lys–Trp (yield 4%) and Lys–Trp (yield 9%) were recovered with the tryptophan intact, and all peptides containing tryptophan or its derivatives isolated from the protein were compatible with this sequence. Amide residues were located by release with exopeptidases or by consideration of the electrophoretic mobility of simple peptides at pH 6.5.

The protein is similar in size to the other c_3 type cytochromes of known structure (table 1), and like them contains four cysteine-histidine clusters. The NMR spectra of cytochromes c_3 [9] are much more complex than those of simple monohaem *c* cytochromes and indicate that the proteins contain at least three and possibly four haem residues per molecule. Recent work (Dr. Terry Meyer, personal communication) in which the haem content of *D. vulgaris* cytochrome c_3 determined from the spectrum of the pyridine haemochromogen indicates that these proteins

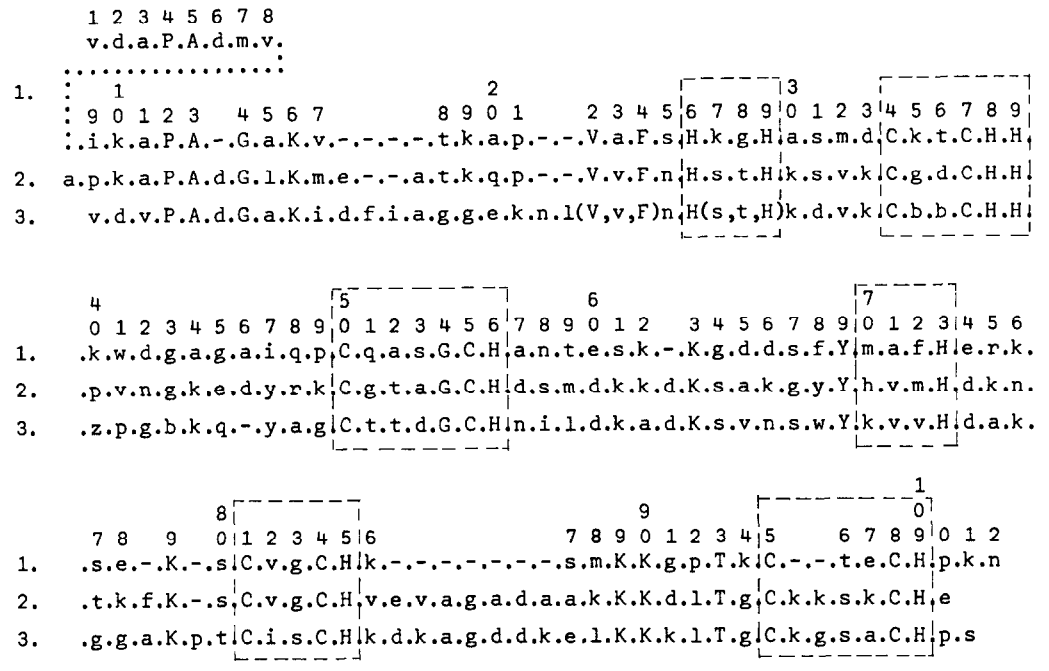

Fig. 2*. Amino acid sequences of cytochromes c_3 from *D. desulfuricans* (1), *D. vulgaris* (2) and *D. gigas* (3) aligned to match best. Residues common to all three proteins are shown in capitals. Numbering is as in fig. 1. The first eight residues of sequence (1) are arranged to show a possible partial gene duplication. The four putative haem-binding sites are boxed, as are the two other histidine-containing regions.

* A one-letter notation recommended by IUPAC-IUB Commission on Biochemical Nomenclature (1968).

probably contain four haem residues per 12,000 M.W., so in the native protein it is very likely that each of these cysteine-histidine clusters binds one haem. The sequences of the three cytochromes c_3 are shown aligned in fig. 2, with insertions and deletions put in to try and maximize the matching. Between residues 22 and 56 the three sequences match very well, with histidine residues at positions 26, 29 and 39 as well as in each of the putative haem binding sites. In the second of these haem sites, (residues 50–56) the cysteine residues are separated by four residues rather than the two found in typical cytochromes *c*. There is less similarity between the other parts of the molecules. The third and fourth haem binding sites are much closer together in the *D. desulfuricans* sequence than in the other two, and in this protein alone the fourth site has the standard two-residue separation for the cysteines. It is possible that the residue at position 70 and histidine-73 may prove to have a similar structural or functional role to the histidines at positions 26 and 29, but proof will require chemical modification experiments or tertiary structure determination. The first eight residues of the *D. desulfuricans* sequence in fig. 2 are arranged to show a possible partial gene duplication.

There is more difference between the sequence of the *D. desulfuricans* protein and that from *D. vulgaris* or *D. gigas* than between the latter proteins though no attempt is made to quantitate this because of the arbitrary value that would have to be given to deletions. This greater evolutionary separation of *D. desulfuricans* is also expressed in the significantly lower G + C content of its DNA (table 1). We await with great interest the sequence of the cytochrome c_3 from *D. salexigens* (G + C = 46%) [3].

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