C-TYPE CYTOCHROMES OF DESULFOVIBRIO VULGARIS

AMINO ACID COMPOSITION AND END GROUPS OF CYTOCHROME C_{553}[x]

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SUMMARY

Cytochrome c_{553} of Desulfovibrio vulgaris differs in amino acid composition and in molecular weight (9,100 ± 100) from cytochrome c_3 (12,000) of the same organism. This protein can be easily obtained in crystalline form. It consists of a single polypeptide chain comprising about 80 amino acid residues and bearing a single heme group.

Both termini are freely available; alanine and leucine are marking the amino terminal and the carboxyl terminal position, respectively.

In addition to cytochrome c_{553} and to cytochrome c_3 a third c-type cytochrome, of larger molecular weight, was detected in D. vulgaris and related organisms.

INTRODUCTION

Postgate in England (1954, 1956) and Ishimoto et al., (1954) in Japan first reported on isolation and properties of cytochrome c_3 from the strict anaerobe Desulfovibrio desulfuricans Hildenborough, now

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renamed D. vulgaris (NCIB 8303) (Postgate and Campbell, 1966), and related sulfate-reducing bacteria. Unlike most c-type cytochromes, cytochrome c_3 contains several heme groups (Ishimoto et al., 1954; Postgate, 1956; Horio and Kamen, 1960; Drucker et al., 1967; Drucker and Campbell, 1969) and exhibits a very low oxidation-reduction potential (Postgate, 1956). Its complete amino acid sequence has already been determined (Ambler, 1968).

Recently, we isolated from the same organism another cytochrome c having the maximum of its reduced α-band also at 553 nm (Le Gall and Bruschi-Heriaud, 1968). The spectra of the two cytochromes are very similar except that cytochrome c_{553} shows a higher peak at 280 nm. Cytochrome c_{553} is further distinguished from cytochrome c_3 by a lower molecular weight, a single heme group and a substantially less negative oxidation-reduction potential (Bruschi-Heriaud and Le Gall, 1967).

In the present communication we report the amino acid composition and the terminal residues of cytochrome c_{553}. This work constitutes the first part of an investigation of the chemical structure of the protein.

EXPERIMENTAL PROCEDURES

Materials

The chemicals used in this study were of reagent grade; they were used without further purification. Carboxypeptidase A-DFP was a 3x crystallized preparation of the Worthington Biochemical Corportion, Freehold, New Jersey. The method used for purification and crystallization of cytochrome c_{553} was that described by Le Gall and Bruschi-Heriaud (1968). After three successive crystallizations, the cytochrome was dialyzed against distilled water and lyophilized.

Amino Acid Analysis

Protein samples (about 0.5 mg) were hydrolyzed in 0.5 ml of 6N HCl at 105-110° for 24 hours. Decomposition of tyrosine and methionine was

almost completely prevented by the addition of thioglycolic acid (0.05%) to the HCl before sealing the tubes. After hydrolysis the HCl was removed under vacuum.

Amino acid analyses were carried out on a modified Beckman-Spinco model 120B amino acid analyzer using the stepwise four buffer elution program of Dus et al. (1966).

Cysteine and methionine were analyzed after performic acid oxidation as cysteic acid and methionine sulfone, respectively (Hirs, 1956). Tryptophan was determined by the method of Spies and Chambers (1949).

Analysis of Amino Terminus

The 1-fluoro-2, 4-dinitrobenzene (FDNB) reaction was performed as described by Sanger (1945, 1949) using 0.5 micromole of protein. The DNP-amino acids were identified by two dimensional chromatography on Whatman N° 1 filter paper. The solvent systems employed were t-amyl alcohol/2N NH_4OH in volume ratios of 4:1 (Murachi and Neurath, 1960) and 1.5 M phosphate buffer, pH 6.0 (Levy, 1954).

Analysis of Carboxyl Terminus

A preparation of DFP-treated carboypeptidase A was used for digestion of native cytochrome c_{553}. The experimental conditions were essentially those outlined by Ambler (1967). The protein was dissolved in 0.2 M N-ethylmorpholine acetate, pH 8.5, and the enzyme was added in a molar ratio of 1:70. The digestion was carried out at 37° for 3 hours. Aliquots of the reaction mixture were withdrawn at appropriate intervals and analyzed on the automatic amino acid analyzer.

The hydrazinolysis procedure of Akabori et al., (1952) was applied using the modification of Niu and Fraenkel-Conrat (1955). The free amino acids were determined quantitatively on the amino acid analyzer.

Determination of Molecular Weight

The approximate size of the protein was determined by gel filtration on a column of Sephadex G-75 following the procedure of Whitaker (1963). The exact molecular weight was calculated from the amino acid composition including one protoheme IX group.

Results

The yield of pure cytochrome c_{553} per kg of wet paste of cells was in the range of 76 mg as compared to a yield of 178 mg of cytochrome c_3 obtained under the same conditions. Cytochrome c_{553} can be readily obtained in crystalline form. Figure 1 shows a crystal of the oxidized heme protein obtained after several recrystallizations in the presence of saturated ammonium sulfate.

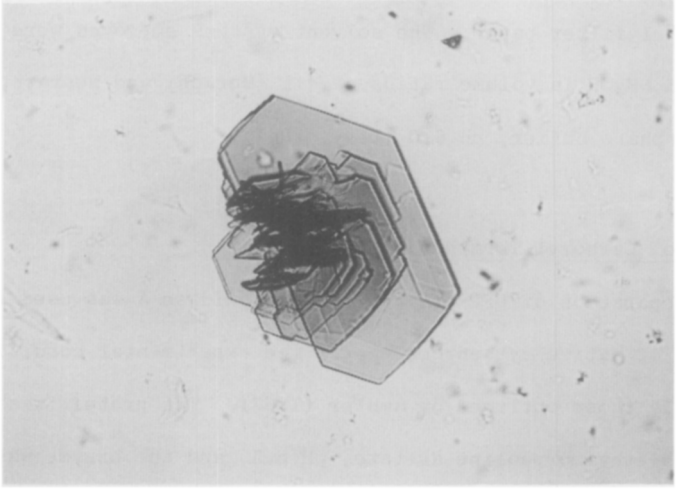

Fig. 1

Crystal of cytochrome c_{553} after three crystallizations. Length of the crystal: approximately 0.2 nm.

Amino Acid Composition

With the exception of tryptophan the amino acid composition given in

Table I

Amino Acid Composition of Cytochrome c_{553}

Cysteic Acid	-	-	1.7	2
Aspartic Acid	6.2	6.3	7.0*	6
Threonine	0.9	2.9	2.8	1
Serine	4.8	4.9	4.0*	6**
Glutamic Acid	6.0	6.0	6.0	6
Proline	1.5	1.9	2.4*	2
Glycine	10.9	10.9	10.5	11
Alanine	12.9	13.0	12.2	13
Valine	1.9	2.0	2.0	2
Methionine	5.2	5.2	-	5
Methionine Sulfone	-	-	4.9	
Isoleucine	0.9	0.9	0.8	1
Leucine	4.9	5.0	4.8	5
Tyrosine	5.9	6.0	3.5*	6
Phenylalanine	trace	trace	-	0
Histidine	1.0	1.0	0.5*	1.
Lysine	12.0	12.0	13.4	12
Arginine	0.9	0.9	1.4*	1
Tryptophan***				0
Total	(1)	(2)	(3)	80

(1) and (2): two separate analyses of hemeprotein in 6 N HCl, 24 hours at 110°;
(3) analysis of performate oxidized protein in 6N HCl, 24 hours at 110°;
*not reliable after oxidation with performic acid;
**extrapolated to zero time hydrolysis;
***determined by the colorimetric method of Spies and Chambers.

Table I was obtained from acid hydrolyzates of the protein before and after performic acid oxidation. Despite the relatively high absorption peak of cytochrome c_{553} around 280 nm, no indication for presence of tryptophan was furnished by colorimetric analysis according to Spies and Chambers (1949). Assuming the presence of a single protoheme IX group the formula weight of cytochrome c_{553} is 9,000. This value agrees very well with the result of the gel filtration experiment which indicated 9,100. However,

for this molecular weight both iron and heme analyses were low (Le Gall and Bruschi-Heriaud, 1968).

Terminal Residues

Amino terminal alanine was found by the FDNB method. No other ether soluble DNP-amino acid was detected. The yields of DNP-alanine, however, were surprisingly low, and no more than 0.4 micromole per micromole of protein was obtained in the best experiments. In order to correct for losses incurred during acid hydrolysis and subsequent paper chromatographic identification, a known amount of synthetic DNP-alanine was heated with 6N HCl at 105-110° for 18 hours in the presence of cytochrome c_{553}. The recovery of DNP-alanine from this step alone was only 60%. Including also the losses sustained in chromatography, a corrected yield of about 75% may be assumed.

From hydrazinolyzates of cytochrome c_{553} leucine was recovered in excellent yields. No other amino acids were noted.

Digestion of native cytochrome c_{553} with carboxypeptidase A for a total period of 3 hrs. yielded mainly leucine and alanine in proportions which suggested the sequence ..... Ala-Leu-OH.

Discussion

From the comparison of cytochrome c_3 and cytochrome c_{553} of D. vulgaris, listed in Table II, it can be seen that these two heme proteins are distinctly different with regard to molecular weight and content of most types of amino acids. Thus, cytochrome c_{553} contains only two residues of cytsteine, just sufficient to link the single heme group to the apoprotein, while cytochrome c_3 has eight residues of cysteine or more than the necessary complement for attachment of even three heme groups. On the other hand, nine residues of histidine are found in cytochrome c_3 but only one in cytochrome c_{553}. On balance, there seem to be at least one

Table II

Comparison of Amino Acid Compositions of

Cytochrome c_3 and Cytochrome c_{553} of D. vulgaris

	Cytochrome c_3 (according to sequence by Ambler, 1968)	Cytochrome c_{553}
Asp	12	6
Thr	5	1
Ser	6	6
Glu	5	6
Pro	4	2
Gly	9	11
Ala	10	13
Cys/2	8	2
Val	8	2
Met	3	5
Ile	0	1
Leu	2	5
Tyr	3	6
Phe	2	0
His	9	1
Lys	20	12
Arg	1	1
Trp	0	0
Total	107	80

histidyl and one methionyl residue available for chelation to each heme iron of either protein as was observed for other cytochromes c (Harbury et al., 1965).

The exact number of heme groups present in cytochrome c_3, namely two or three, is still at issue (Ishimoto et al., 1954; Postgate, 1956; Horio and Kamen, 1960; Drucker et al., 1967; Drucker and Campbell, 1969). It is interesting that the amino acid sequence of cytochrome c_3 (Ambler,

1968) revealed only two sequences of the type CyS-X-Y-CyS-His, characteristic of the heme attachment sites in c-type cytochromes. The protein contains, however, two additional sequences of the type CyS-A-B-C-D-Cys-His which strikingly differ from the normal arrangement.

In contrast to cytochrome c_3, cytochrome c_{553} has only a single heme group and all the makings of a typical c-type heme attachment.

To underscore the differences between cytochrome c_3 and cytochrome c_{553} a comparison of the two proteins is given in Table III.

Availability of several soluble c-type cytochromes from the same organism, D. vulgaris, in reasonably good yields, is very intriguing

Table III

Properties of c-type Cytochromes of D. vulgaris:

	Cytochrome c_3	Cytochrome c_{553}
Amino Acid Residues	107	80
Heme Groups	2-3*	1
Molecular Weight	12,000	9,000
Amino Terminal Residue	Ala	Ala
Carboxyl Terminal Residue	Glu	Leu
Midpoint Potential	-205mV	between -100 and OmV
Isoelectric Point	10.5	basic ($>$8.6)

* See references: Ishimoto et al., 1954
Postgate, 1956
Horio and Kamen, 1960
Drucker et al., 1967

especially in view of the apparently rudimentary and still poorly understood electron transport system of the sulfate-reducing bacteria. For instance, in D. vulgaris, D. gigas, and D. desulfuricans we have detected the presence of yet another cytochrome c which has a molecular weight of about 26,000. Several of its other properties are also different from those of either cytochrome c_3 or cytochrome c_{553}. The physiological functions of both cytochrome c_{553} and the larger c-type cytochrome are still unknown.

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