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SEQUENCE HOMOLOGY OF BACTERIAL AND MITOCHONDRIAL CYTOCHROME C OXIDASES

Partial sequence data of cytochrome c oxidase from Paracoccus denitrificans

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SUMMARY: The aerobic electron transport chain of Paracoccus denitrificans is very similar to that of mitochondria. It has therefore been suggested that this bacterium might be evolutionarily related to mitochondria. The two subunits (Mr 45.000 and 28.000) of the Paracoccus cytochrome c oxidase were isolated and partially sequenced. The sequences were found to be surprisingly homologous to sequences of the subunits I and II of mitochondrial cytochrome c oxidases. The data provide a molecular basis for the symbiotic origin of mitochondria and strongly support the notion that in eucaryotic oxidases subunits I and/or II carry the redox centers, heme and copper.

INTRODUCTION: The electron transport chain of the Gram-negative bacterium Paracoccus denitrificans shows striking functional similarities to that of mitochondria (1), suggesting a common ancestry of this bacterium and the organelle.

Thus it was recently shown that cytochrome c oxidase from this bacterium closely resembles the mitochondrial enzyme in many spectral and kinetic properties (2). The structure of this procaryotic oxidase, however, is much simpler: only two polypeptides with apparent Mr 45.000 and 28.000 are present (3) as compared to the twelve polypeptide pattern of the mammalian type cytochrome c oxidase (respiratory complex IV) (4). The bacterial

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Abbreviations used: SDS Sodiumdodecylsulfate
CNBr Cyanogenbromide
PAGE Polyacrylamide gel electrophoresis

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oxidase is fully functional in electron transport and also acts as a redox-driven proton pump (5). Immunological studies have already revealed common determinants on subunit II of Paracoccus, beef heart and yeast oxidases (2).

The present study reports the determination of partial sequences of the two subunits of Paracoccus oxidase and a comparison with corresponding sequences of eucaryotic oxidases.

MATERIALS AND METHODS: Cytochrome c oxidase from the cytoplasmic membrane of Paracoccus denitrificans was prepared as described earlier (3).

Isolation of the subunits I and II was performed by dissociation of the oxidase in 3% SDS, 5% β-mercaptoethanol at 37°C and subsequent chromatography on Bio-Gel P200 (minus 400 mesh) in 3% SDS. Eluted fractions were pooled after analytical SDS PAGE (6) and lyophilized. Excess SDS was removed by applying the isolated subunits on a Bio-Gel P6 column equilibrated with 0.01% SDS. The protein material was then lyophilized.

Cleavage with CNBr was performed as described by Gross & Witkop (7).

Chromatography of the CNBr-fragments was carried out on Bio-Gel P10 columns in 70% acetic acid. Protein peaks were identified by their absorbance at 280 nm or after alkaline hydrolysis and subsequent ninhydrin staining. If required, CNBr-fragments were rechromatographed on Bio-Gel P30 in 70% acetic acid.

Carboxymethylation of the isolated subunits was performed essentially as described by Crestfield et al. (8). The protein material was separated from the reagents by chromatography on Bio-Gel P30 in 80% formic acid.

Amino acid analysis was performed as described in (9). Cysteine was determined as carboxymethylcysteine.

Automatic Edman degradation was carried out on a Beckman 890C Sequencer as previously described (10). Identification of the phenylthiohydantoin amino acids was done by HPLC on reverse phase columns with a Spectra-Physics SP 8000.

RESULTS AND DISCUSSION: The two subunits of the Paracoccus cytochrome c oxidase of Mr 45.000 and 28.000 can be separated on a preparative scale by gel filtration on Bio-Gel P200 in 3% SDS. The amino acid compositions of both subunits given in Table I agree well with those reported earlier (3) with some differences in the values for methionine and tyrosine.

Table I includes the polarity indices of the two subunits, valued of 33 and 39 are obtained for subunit I and subunit II,

TABLE I
Amino acid analyses of carboxymethylated subunits of cytochrome c oxidase from Paracoccus denitrificans

	Subunit I mol %	Subunit II mol %
Asx	7,2	10,9
Thr	5,8	3,6
Ser	5,0	3,6
Glx	5,7	11,9
Pro	5,7	7,0
Gly	11,6	6,1
Ala	9,9	13,6
Val	6,4	10,3
Met	4,6	1,7
Ile	5,6	4,9
Leu	9,8	10,6
Tyr	4,4	3,5
Phe	7,9	4,7
His	4,2	2,6
Lys	2,2	3,6
Arg	3,0	2,7
Cys*)	0,54	0,74
Trp	n.d.	n.d.
Polarity index	32,99	39,08

*) The values for cysteine were obtained from the sum of cysteic acid and carboxymethylcysteine

resp. Both values are considerably lower than the corresponding values of the beef heart oxidase (35.7 and 44.7, resp.) (11,12).

The cysteine content of subunit I (0,54 mol %) leads, on the basis of an apparent molecular weight of 45.000 to the assumption of 2-3 cysteine residues, whereas the cysteine content of subunit II (0,74 mol %) on the basis of a Mr of 28.000, is close to a number of 2 cysteine residues. The values for cysteine often being underestimated, 3 and 2 cysteine residues are likely to be present in subunits I and II, resp.

In an attempt to obtain N-terminal sequences for both subunits, the isolated polypeptide chains were subjected to automatic Edman degradation using the quadrol protein program (13). However, no phenylthiohydantoin derivatives could be obtained in an amount corresponding to the polypeptide mass present. After performing a deformylation procedure according to (14), the

TABLE II

Partial sequences of CNBr-fragments of subunits I and II of Paracoccus denitrificans cytochrome c oxidase. Alignment with corresponding sequences of yeast (16,17), maize (15) and beef heart oxidase (11,12)

		92
Beef Heart	I	-Met-Ala-Phe-Pro-Arg-Met-Asn-Asn-Met-Ser-Phe-
Yeast	I	-Thr-Ala-Phe-Pro-Arg-Ile-Asn-Asn-Ile-Ala-Phe-
Paracoccus	I	-Met-Ala-Phe-Pro-Arg-Leu-Asn-Asn-Leu(Ser)Tyr-
		277
Beef Heart	I	-Met-Met-Ser-Ile-Gly-Phe-Leu-Gly-Phe-Ile-Val-Trp-Ala-His-
Yeast	I	-Met-Ala-Ser-Ile-Gly-Leu-Leu-Gly-Phe-Leu-Val-Trp-Ser-His-
Paracoccus	I	-Met-Ala-Ala-Ile-Gly-Ile-Leu-Gly-Phe-Val-Val-Trp-Ala-His-
		207
Beef Heart	II	-Met-Pro-Ile-Val-Leu-Glu-Leu-Val-Pro-Leu-Lys-Tyr-Phe-Glu-Lys-Trp-Ser-Ala-Ser-
Yeast	II	-Met-Pro-Ile-Lys-Ile-Glu-Ala-Val-Ser-Leu-Pro-Lys-Phe-Glu-Leu-Trp-Leu-Asn-Glu-
Maize	II	-Thr-Pro-Ile-Val-Val-Glu-Ala-Val-Thr-Leu-Lys-Asp-Tyr-Ala-Asp-Trp-Val-Ser-Asn-
Paracoccus	II	-Met-Pro-Ile-Val-Val-Lys-Ala-Val-Ser-Gln-Glu-Lys-Tyr-Glu-Ala-Trp-Leu-Ala-Gly-
		89
Beef Heart	II	-Glu-Ile-Asn- -Asn-Pro-Ser-Leu-Thr-Val-Lys-Thr-Met-Gly-His-Gln-Trp-Tyr-Trp-Ser-Tyr-Glu-Tyr-
Yeast	II	-Glu-Val-Ile- -Ser-Pro-Ala-Ile-Thr-Ile-Lys-Ala-Ile-Gly-Tyr-Gln-Trp-Tyr-Trp-Lys-Tyr-Glu-Tyr-
Maize	II	-Gly-Val-Leu-Val-Asp-Pro-Ala-Ile-Thr-Ile-Lys-Ala-Ile-Gly-His-Gln-Trp-Tyr-Trp-Ser-Tyr-Glu-Tyr-
Paracoccus	II	-Met-Pro-Asn- -Asp-Pro-Asp-Leu-Val-Ile-Lys-Ala-Ile-Gly-His-Gln-Trp-Tyr-Trp-Ser-Tyr-Glu-Tyr-

same negative results were obtained. We therefore conclude that both polypeptides are N-terminally blocked. The presence of formyl-methionine as a blocking group as in the beef heart oxidase is, however, unlikely.

After CNBr-cleavage of subunit I, in agreement with the amino acid composition, more than 10 CNBr-fragments could be isolated. So far the amino acid sequences of two of these fragments were determined. The results are given in Table II. The comparison with the corresponding sequences of yeast and beef heart oxidase clearly shows that the partial sequences of subunit I of Paracoccus denitrificans are highly homologous to the corresponding sequences of the mitochondrial oxidases. 15 out of 25 residues in the given examples are invariant; all these exchanges are of conservative nature.

In the case of subunit II, in accordance with the relatively low methionine content, we so far have identified 5 CNBr-fragments. Partial sequences of two of them are given in Table II. As in the case of subunit I, the sequences can easily be aligned to known sequences of subunit II of beef heart (11,12), maize (15) and yeast (16) cytochrome c oxidase.

Besides a number of invariant or conserved residues the substitution pattern here shows the introduction of charged residues or changes of the polarity. Subunit II is the binding site for cytochrome c in eucaryotic oxidases and in the Paracoccus oxidase (18,19). Changes in the distribution of charged amino acids may reflect the structural adaptations to different cytochromes c (eucaryotic and Paracoccus cytochrome c) (20). The cluster of aromatic residues -Trp-Tyr-Trp-Ser-Tyr-Glu-Tyr- included in beef heart sequence positions 104-110 (Table II) is completely invariant. This special structure has already been suggested to be involved in electron transfer between the metal centers of the enzyme (21).

These partial sequences prove that subunits I and II of Paracoccus cytochrome c oxidase are homologous to the two largest subunits of the more complex mitochondrial enzymes and indicate that also in the mitochondrial oxidases the four redox centers are located in subunits I and/or II. It is unclear at present, whether subunit III, a polypeptide coded for by the mitochondrion, has any counterpart or corresponding gene in Paracoccus denitrificans, since a third subunit is not present in its fully functional oxidase complex.

Finally the presented data provide a contribution from molecular biology to the symbiotic origin of mitochondria (1,22). Küntzel and Köchel (22) in their comparison of rRNA's refered

to E. coli, whereas in our case the comparison has been done with a bacterium much more closely related to mitochondria.

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