

A gene in *Paracoccus* for subunit III of cytochrome oxidase

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The region of *Paracoccus denitrificans* chromosome where the genes coding for cytochrome oxidase (cytochrome *aa₃*) subunits are located has been cloned. DNA sequencing revealed an open reading frame that codes for a protein homologous to the subunit III of the eukaryotic, mitochondrial enzyme. This subunit is absent from the isolated *Paracoccus* oxidase. It now seems that it is part of the native enzyme in the bacterial cytoplasmic membrane. This may explain the observed discrepancies in the function of the isolated enzyme.

Cytochrome oxidase Subunit III (Paracoccus) DNA sequencing

1. INTRODUCTION

Aerobic bacteria are able to use many different terminal oxidases in cellular respiration. One of these, cytochrome *aa₃*, is structurally and functionally similar to the mitochondrial enzyme [1,2]. This enzyme catalyses electron transfer from cytochrome *c* to dioxygen and coupled to this transfers two protons/electron across the membrane, one of which is used in formation of water [3]. The bacterial oxidase has been isolated from a number of species and shown to contain 2 or 3 different subunits. These correspond to the functionally central subunits I, II and III in the mitochondrial enzyme [4] that are coded for by three genes in the mitochondrial DNA itself; the subunits coded for by nuclear genes appear to be absent in the bacterial enzymes [1–3].

The first known bacterial cytochrome *aa₃* was purified by Ludwig and Schatz [5] from *Paracoccus denitrificans*. The isolated enzyme contains two different subunits which are known to be homologous to the subunits I and II of the mitochondrial enzyme [2,6]. It lacks, however, the third subunit. In mitochondria and some other bacteria [1,3] this subunit III is retained in the isolated oxidase. It seems to be important in proton translocation, directly or indirectly. Dicyclohexylcarbodiimide (DCCD) modifies a

glutamic acid residue [7] residing in one of its membrane-spanning segments [4]. This leads to inhibition of net proton translocation while electron transfer is not greatly affected [8].

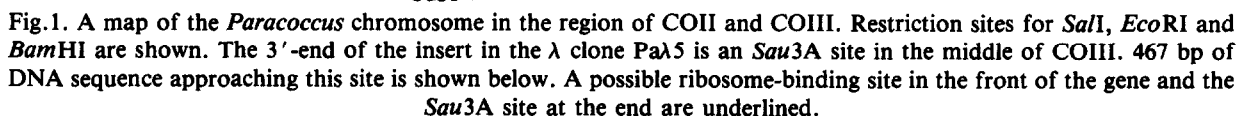
It has been difficult to demonstrate effective proton translocation with the purified *Paracoccus* cytochrome oxidase reconstituted to liposome membranes [9]. The enzyme in situ clearly has this activity [10]. We have been cloning the genes of the *Paracoccus* cytochrome oxidase. Here, we have now found a gene in the genomic DNA that codes for the subunit III. This subunit must be lost during the traditional isolation procedure [5], and its loss renders the isolated enzyme a poor proton translocator as seems to be the case for the mitochondrial oxidase in the equivalent situation [11,12].

2. MATERIALS AND METHODS

P. denitrificans S1657 was a gift from Dr Henk van Verseveld. A DNA library was constructed by cloning partially *Sau*3A-digested DNA fragments with the λ EMBL-3 vector in *E. coli* NM539. The library was screened with two synthetic oligonucleotide probes (see below). DNA hybridization, isolation of λ DNA and restriction mapping is described in [13]. *Sal*I restriction fragments hybridizing to the probes were purified

The DCCD-binding site in subunit III has been identified by Prochaska et al. [7]. It is a glutamic

Two synthetic oligonucleotides were designed as mixed probes on the basis of the evolutionary conservation of the protein sequence [4] and the known peptide sequences of the *Paracoccus* proteins [6]. They were both directed to the gene of subunit II. Hybridization was carried out with two probes in parallel, and λ clones positive with both were selected for further analysis by restriction mapping and DNA sequencing. The gene for the subunit II was first found by sequencing the ends of two *SalI* fragments shown in fig.1. The figure gives a rough map of the *Paracoccus* chromosome in the region of the subunit II and III genes (COII



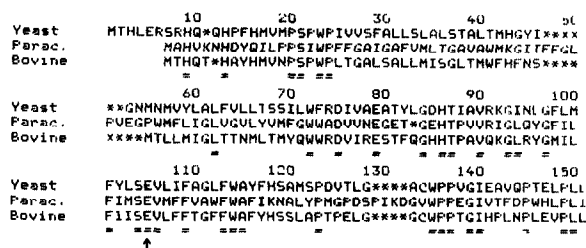


Fig. 2. Alignment of N-terminal amino acid sequences of bovine [20], *Saccharomyces* [21] and *Paracoccus* subunit III. Identical residues in all three are underlined. The arrow points to the DCCD-binding glutamic acid.

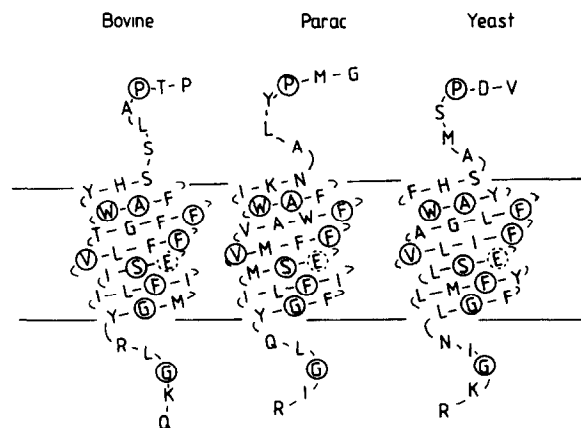


Fig. 3. Transmembrane segment containing the DCCD-binding site in yeast, bovine and *Paracoccus* subunit III. Identical residues are circled, and the DCCD-binding site is indicated with a dashed circle.

acid residue inserted into a long hydrophobic segment, one of the proposed transmembrane helices in this protein [4]. This glutamic acid is present in the *Paracoccus* protein as well (dashed circle in fig.3). Conservation of the amino acid sequence shows helical geometry in this segment: one side of the predicted helix has an array of invariant amino acids, notably aromatic residues (circled in fig.3).

We conclude that subunit III is probably present in the *Paracoccus* cytochrome oxidase as it is found in some other bacterial cytochromes *aa₃* [1,18]. Alternative purification procedures [19] may yield preparations that retain this subunit. Its absence from the conventional preparation is likely to be the reason why the reconstituted enzyme shows a low proton/electron transport stoichiometry [9,12].

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