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The cytochrome *c* peroxidase of *Paracoccus denitrificans*

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The size, visible absorption spectra, nature of haem and haem content suggest that the cytochrome *c* peroxidase of *Paracoccus denitrificans* is related to that of *Pseudomonas aeruginosa*. However, the *Paracoccus* enzyme shows a preference for cytochrome *c* donors with a positively charged 'front surface' and in this respect resembles the cytochrome *c* peroxidase from *Saccharomyces cerevisiae*. *Paracoccus* cytochrome *c*-550 is the best electron donor tested and, in spite of an acidic isoelectric point, has a markedly asymmetric charge distribution with a strongly positive 'front face'. Mitochondrial cytochromes *c* have a much less pronounced charge asymmetry but are basic overall. This difference between cytochrome *c*-550 and mitochondrial cytochrome *c* may reflect subtle differences in their electron transport roles. A dendrogram of cytochrome c_1 sequences shows that *Rhodopseudomonas viridis* is a closer relative of mitochondria than is *Pa. denitrificans*. Perhaps a mitochondrial-type cytochrome *c* peroxidase may be found in such an organism.

Introduction

The bacterium, *Paracoccus denitrificans*, is an important model system for bioenergetic studies and has been proposed to be a close evolutionary relative of the eukaryotic mitochondrion [1]. *Pa. denitrificans* produces a variety of different *c*-type cytochromes, depending on the growth conditions [2,3]. We have shown that one of these cytochromes is a cytochrome *c* peroxidase of approx. M_r 42 000 [4]. This paper compares this enzyme with the well-characterised cytochrome *c* peroxidases of *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* and discusses the role of the enzyme in the electron transport system of *Pa. denitrificans*.

The molecular properties of *Paracoccus* denitrificans cytochrome *c* peroxidase

The visible spectra of *Pa. denitrificans* cytochrome *c* peroxidase closely resemble those of the *Pseudomonas aeruginosa* enzyme [4]. They share the distinctive features of well-separated Soret bands, a split α-band in the reduced state, and a small high-spin component absorbing near 630 nm in the oxidised state.

The relative molecular mass of the *Pa. denitrificans* enzyme in SDS was 42 000 and this was not altered by removal of the haem [4]. The amino acid composition expressed in proportion to 2 mol haem gave $M_r \approx 34\,000$. The discrepancy between these two figures has not been resolved, but reflects a similar discrepancy found for the *Ps. aeruginosa* enzyme, the sequence of which has been determined [5]. We conclude that both enzymes have two haem *c* groups.

The *Pa. denitrificans* cytochrome *c* peroxidase therefore resembles the *Ps. aeruginosa* enzyme with respect to size, spectra, nature of haem and haem content. In view of these similarities we propose that the mechanism of the *Paracoccus* enzyme is likely to follow that proposed for the *Pseudomonas* enzyme. This involves the two-electron reduction of hydrogen peroxide bound to the peroxidatic haem. One electron is donated from the peroxidatic iron itself and the second from the second haem group [6].

The specificity of *Paracoccus* cytochrome *c* peroxidase and its role in the electron transport system

Although the *Paracoccus* enzyme resembles the *Pseudomonas* enzyme in these respects it differs in its specificity for the electron-donating cytochrome *c*. The *Pseudomonas* enzyme shows a strong preference for the acidic cytochromes *c*-551 and oxidises the basic mito-

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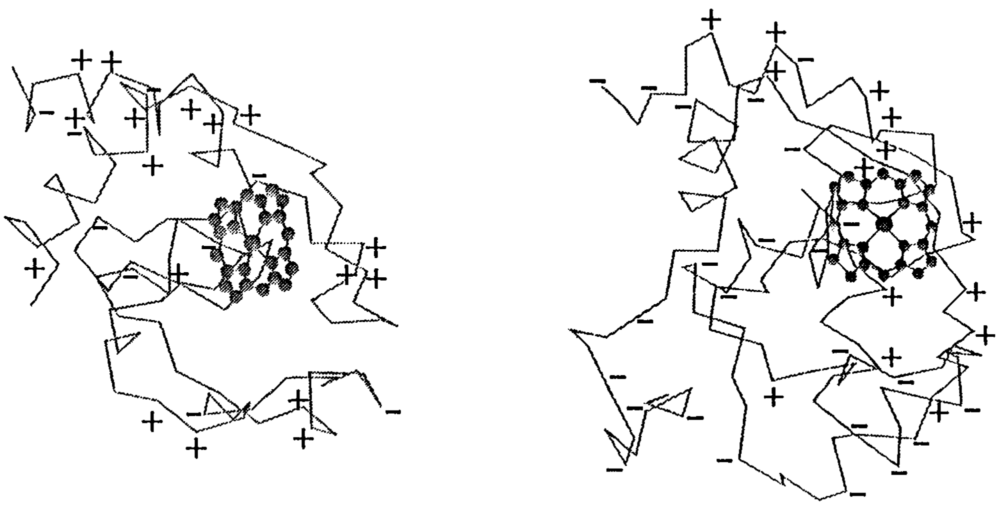

Tuna cytochrome c - side

Pa.denitrificans cytochrome c-550 - side

Fig. 1. The surface charge distribution of tuna cytochrome *c* and *Paracoccus denitrificans* cytochrome *c*-550. The X-ray crystallographic structures are from Ref. 12 (tuna) and Ref. 13 (*Pa. denitrificans*). The views are of the left side of the molecules with the 'front face' and the exposed haem edge to the right. Charges are placed at the α-carbon positions of Lys, Arg, Asp and Glu residues.

chondrial cytochromes *c* only poorly. The opposite pattern was observed for the *Paracoccus* enzyme and in this respect it therefore resembles *S. cerevisiae* cytochrome *c* peroxidase. However, the cytochrome *c*-550 of *Pa. denitrificans* was the best donor of all to the *Paracoccus* enzyme and yet it has an acidic isoelectric point [4].

This apparent anomaly is resolved if the charge distribution on mitochondrial cytochrome *c* and *Paracoccus* cytochrome *c*-550 is considered (Fig. 1). The latter has a much more pronounced asymmetry of charge distribution which results in a strongly positive 'front face' in spite of the excess of overall negative charge in the molecule. Since it is this surface that has been identified as the route of electron transfer, we propose that the preference of the *Paracoccus* cytochrome *c* peroxidase is for cytochromes *c* with a positive 'front face' rather than particularly for those with an overall positive charge.

It has been proposed [7] that the molecular dipole of mitochondrial cytochrome *c* is instrumental in an electrostatic pre-orientation of the molecule and its redox partners, thus enhancing fruitful collision. Such a model seems even more applicable to *Paracoccus* cytochrome *c*-550, with its much more pronounced molecular dipole (Fig. 1). This may reflect a relative simplicity in the role of *Paracoccus* cytochrome *c*-550 compared to its mitochondrial counterpart. The latter must perform two electron-transfer functions. It must diffuse rapidly across the inner membrane surface to mediate electron transfer between Complex III and cytochrome *c* oxidase. It must also diffuse within the intermembrane space to interact with such enzymes as cytochrome *c* peroxidase. In contrast, in *Pa. denitrificans*, the equivalent to the former function is performed by a membrane-bound cytochrome *c*-552 [8] leaving the cytochrome *c*-550 the role of mediating electron transfer between the mem-

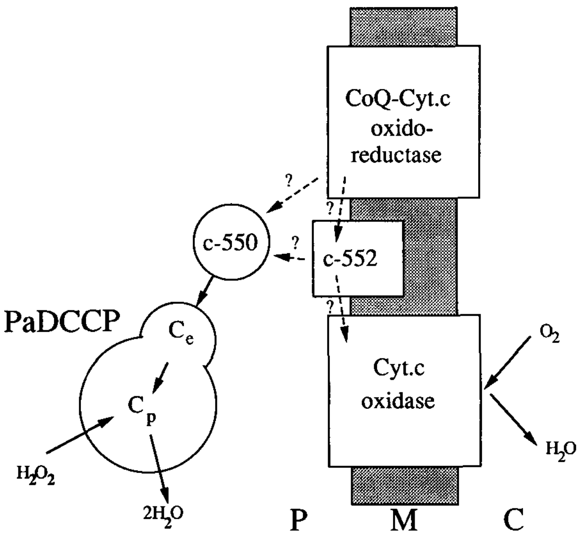

Fig. 2. The electron transport system of *Paracoccus denitrificans*. The scheme shows the respiratory complexes, CoQ-cytochrome *c* oxidoreductase and cytochrome *c* oxidase embedded in the bacterial cell membrane and linked via the membrane-bound cytochrome *c*-552. Cytochrome *c*-550 and cytochrome *c* peroxidase (PaDCCP) are periplasmic (P) components. The proposed electron transferring (C_e) and peroxidatic domains (C_p) of PaDCCP are shown.

brane and soluble periplasmic enzymes such as cytochrome *c* peroxidase (Fig. 2).

We propose that the simple molecular dipole of cytochrome *c*-550 is suited to this role of free diffusion and collision while the dual role required of mitochondrial cytochrome *c* involving the additional feature of lateral diffusion across a negatively charged membrane surface requires a more subtle surface charge distribution.

The evolutionary origins of the mitochondrion

In our studies of bacterial cytochrome *c* peroxidases we had anticipated the occurrence of the yeast-type enzyme in organisms like *Pa. denitrificans*. Although we have characterised an enzyme resembling that of yeast in specificity, its molecular properties are those of the *Pseudomonas* enzyme. We may ask whether the yeast enzyme will have a bacterial counterpart.

Pa. denitrificans is, of course, often considered a likely relative of the mitochondrion, but a dendrogram of the cytochrome c_2 sequences seems to show that it might not be the closest extant relative [9]. According to this analysis, mitochondrial cytochromes *c* originate within a subgroup of cytochromes c_2 which includes both photosynthetic organisms such as *Rhodopseudomonas viridis* and non-photosynthetic relatives such as *Nitrobacter agilis*. *Pa. denitrificans* occupies a different part of the tree. However, the dendrogram of the cytochromes c_2 sequences shows ambiguities for remote branch points, probably due to the problem of saturation [10].

In contrast, the cytochrome c_1 tree gives a clearer picture in this respect (at least with the small number of sequences so far determined). We have fitted the recently determined cytochrome c_1 sequence of *Rps. viridis* [11] to a dendrogram [9] and find that it does indeed appear to lie closer to mitochondrial cytochromes c_1 than to the group containing *Pa. denitrificans* (Fig. 3). It may be that *Rps. viridis* or its non-photosynthetic relatives harbour a cytochrome *c* peroxidase resembling that of yeast mitochondria.

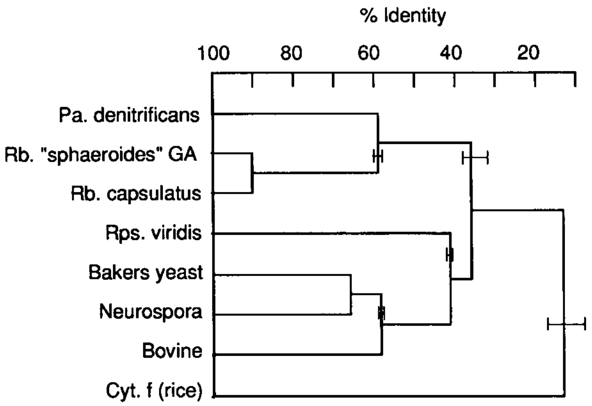

Fig. 3. A dendrogram of the cytochrome c_1 sequences. The dendrogram was constructed as described in Ref. 9 from the known cytochrome c_1 sequences. To this was added the recently determined sequence of cytochrome c_1 from *Rhodopseudomonas viridis* [11].

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