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Commentary

The similarity of cytochrome *c* and ubiquinol oxidases

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A number of terminal H^+-translocating cytochrome *c* and ubiquinol oxidases have homologous primary structures and analogous prosthetic groups and are generally thought to form a superfamily of mechanistically similar enzymes. In contrast, Musser et al. [1] have recently emphasized the differences between the two subclasses, suggesting that these are likely to manifest themselves in distinct electron transfer and proton translocation mechanisms.

While Musser et al. [1] agree that the homologies between subunits I do suggest similar O_2 reduction mechanisms, they argue that the low sequence identity of subunits II and the absence of the Cu_A site in subunit II of the quinol oxidases could lead to different structures and to different electron transfer mechanisms. The question then is, how different the folded structures of the different subunits II actually are?

Despite the low degree of sequence identity, the hydropathy profiles of subunits II are similar, and both are predicted to have similar secondary structures [2,3]. But perhaps the most specific piece of evidence for their structural similarity is this: replacing six residues of the quinol oxidase subunit II with those found in the corresponding cytochrome *c* oxidase subunit creates a copper site similar to Cu_A [4]. This strongly suggests similar overall folding patterns for subunits II. Two factors may contribute to the low degree of sequence conservation in subunit II: its location on the surface of the complex (residues facing the solvent generally show more variability) and the possible location of the quinol binding site in subunit II or at the intersubunit interface.

Copper was not available in the biosphere until oxygen appeared [5]. It is therefore conceivable that the ubiquinol oxidase which has only one copper site could represent the more ancient form, which appeared already when $[O_2]$ was relatively low. The Cu_A site in subunit II appeared later when more Cu(II) had became available due to more oxidizing conditions. An important functional consequence of the Cu_A site is that cytochrome *c* oxidases pump protons using about 180 mV narrower redox span than the quinol oxidases. In other words, ubiquinol oxidases are likely to have a less efficient, and probably simpler, electron gating mechanism than cytochrome *c* oxidases, in contrast to the complex mechanism proposed in [1]. Furthermore, the assumption that the Cu_A containing cytochrome *c* oxidase evolved from a quinol oxidase leads one to think that the true functional core structure of both types of enzyme is made up by subunits I and II, and that subunit II played a role prior to the acquisition of the Cu_A-site. One possible role of subunit II is a structural one[1]. In fact, calorimetric studies show that subunits I and II of a cytochrome *c* oxidase interact strongly in a redox-dependent manner (T. Haltia, N. Semo and E. Freire, unpublished data). Recent structural models suggest that the active site in subunit I might be close to the intersubunit surface [6,7]. Considering the above, proton translocation models involving only the binuclear center in subunit I leave a minor role for the protein structure in controlling the electron transfer and may thus be oversimplified, especially if the quinol binding site is in subunit II. In general, that less than 10% of the residues are invariant within the family suggests two possibilities: either the proton-electron linkage is very direct and an immediate result of the redox chemistry at one of the metal centers in subunit I; or there is, in addition to the metal centers in subunit I, a critical conserved parameter of a more global nature, e.g. a hydrogen bonding pattern or a network of water molecules. In order to define the core structure of H^+-translocating heme-copper oxidases more accurately, it would be of utmost importance to know whether the

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[1]For this function the correctness of the above evolutionary hypothesis is, of course, not crucial. For a discussion and perhaps another view on the origin of the *E. coli* cytochrome *bo* ubiquinol oxidase, see [19].

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novel (and the most ancient?) cytochrome *c* oxidase described in [8] is a proton pump.

Musser et al. [1] propose that electron input and proton translocation by a quinol oxidase occurs by a Q-loop mechanism, which involves a branched electron transfer pathway with a direct electron transfer from the quinol to the O_2 reduction site. Their reasoning about the high potential of the ubiquinol/ubisemiquinone couple being the basis for the branched mechanism may not be valid, however, as the enzyme may lower the potential by stabilizing the semiquinone. In addition, most studies suggest that the low spin heme *b* has a significantly higher potential than 60 mV [9–11]. Moreover, interpretation of redox titration data may be complicated (cf. [11]) because the low spin site in the oxidase from the overexpressing strain used in some studies appears to be occupied in part by heme O (which may have a lower E_m than protoporphyrin IX normally in the site) [12]. According to the model shown in Fig. 2 in [1], there is no electron transfer from the low spin heme to the O_2 reducing site in a quinol oxidase. It should be noted that this is not supported by published data [6,13,14] and, if true, would probably lead to differences in O_2 reduction chemistry. However, despite the unlikely nature of the Q-loop mechanism, it is interesting to note that for cytochrome *c* oxidase a (different) branched electron transfer pathway from Cu_A ($Cu_A \rightarrow$ low spin heme $\rightarrow$ the O_2 reducing site and $Cu_A \rightarrow$ the O_2 binding site directly) has been suggested [15].

It is clear that one should not accept the mechanistic similarity (or dissimilarity) of cytochrome *c* and quinol oxidases without experimental evidence. As Musser et al. point out [1], dogmatism should be avoided. However, given the sequence and functional homologies (and the short time that has passed since the existence of the oxidase superfamily was recognized), it makes sense to pose questions in the framework of earlier results of oxidase studies and ask, for example: Could a branched electron transfer pathway produce the as yet unexplained biphasic cytochrome *c* oxidation kinetics [16]? Does a ubiquinol oxidase exhibit biphasic kinetics? How to explain the anomalous results obtained with the quinol analogue 2-heptyl-4-hydroxyquinoline *N*-oxide [11]? Do ubiquinol oxidases have a higher apparent K_m for oxygen than cytochrome *c* oxidases? (Reported values range from 0.05 μM for the mitochondrial cytochrome *c* oxidase [17] to 2.9 μM for *E. coli* ubiquinol oxidase [18], but data may not have been obtained under comparable conditions.) If a difference would be observed, would it owe to the absence of Cu_A or to structural differences in the O_2 reducing site? Can a ubiquinol oxidase be converted into a cytochrome *c* oxidase (or vice versa)?

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