BIOENERGETICS

# Purpose of proton pathways

*R. J. P. Williams*

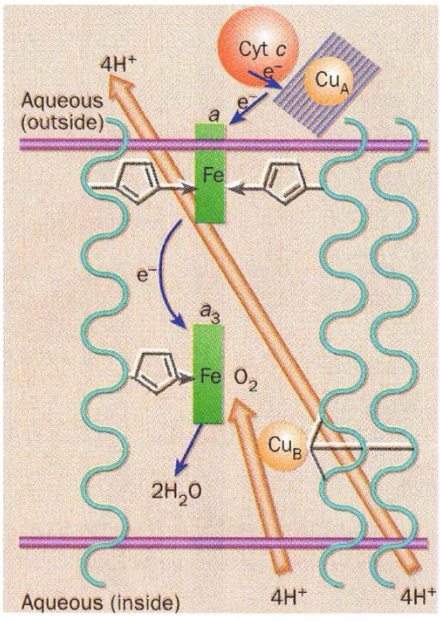
STEP by step, the features of energy capture and its transduction are being revealed. For the respiratory chain in bacterial membranes, this quest has culminated in the announcement by Michel and colleagues on page 660 of this issue[1] of the structure of the enzyme cytochrome oxidase, the last component of the chain.

The respiratory chain harnesses the energy released by electrons derived from metabolic processes as they are transferred along the chain, using it to make a proton gradient and the ATP needed for activity inside the cell. Cytochrome oxidase is the enzyme that catalyses the last step in the respiratory chain, namely the transfer of electrons to molecular oxygen, which is converted to water, using and energizing protons in the process.

Michel and colleagues describe the structure of cytochrome oxidase from the soil bacterium *Paracoccus dinitrificans*[1]. Before turning to the significance of the structure, two points deserve to be highlighted. First, great skill has been shown in obtaining crystals of a transmembrane protein, achieved by using an antibody complex to stabilize the protein in a lattice. Second, the structure uncovers the manner in which the four membrane-bound subunits of cytochrome oxidase are held together. The functions of two of these are unknown, but subunits III and IV use their transmembrane helices to bind the active subunits I and II; subunit III also binds a lipid molecule. Hence this structure, together with that of the bacterial reaction centre, also solved by Michel and colleagues[2], opens up the prospect of reaching an understanding of (membrane) protein assembly.

Michel *et al.* discuss the function of cytochrome oxidase in the context of its structure. This is to take electrons from the penultimate electron carrier, cytochrome *c*, at a redox potential of around 250 mV, and to pass them to the dioxygen reaction site in the membrane, which has a redox potential of 800 mV (ref. 3). In the course of this reaction, which requires four protons, four additional protons (called pumped protons; ref. 4 and references therein) are energized by transfer across the membrane, so generating transduction to the full proton gradient. The figure summarizes what can be deduced from the structure in the light of earlier models.

The crystal structure shows that active subunits II and I are respectively a copper ($Cu_A$)-containing protein β-barrel and a helical transmembrane protein, which carries the two haem units and another copper ($Cu_B$) (see figure). Subunit II is an unusual blue copper protein: it has two copper ions, not one, at the entatic site[3] and its sequence is extended by two transmembrane helices which locate it in the membrane structure. It also has a suitable aqueous-phase binding site for cytochrome *c*. Subunit I has seven transmembrane helices and holds all three electron-transfer centres remarkably close together, with one, haem *a*, at less than 15 Å from $Cu_A$ and 4.7 Å from haem $a_3$. The other two electron-transfer centres have a metal-to-metal distance of 5.2 Å. Note,




The proposed outline structure of cytochrome oxidase (modified from ref. 4). Thin arrows show the electron paths and the thick arrows the proton paths[3]. Histidine residues bound to iron are shown in grey; three more bind to $Cu_B$ (see text).

however, that the $Cu_B$-coordinated ligands and the $O_2$ pocket are not well defined at this resolution. All three of these sites are much closer to the outside surface of the membrane than expected (see figure).

The electron-transfer rate, given the short distances and the large favourable driving forces[5], must be much faster than the turnover of the enzyme at $10^3$ per second. There is clearly no requirement for special through-bond electron transfer, but there are puzzles. Why have a $Cu_A$ site with two copper atoms? Although this structure of the inhibited oxidized state delineates a possible site for dioxygen, does it give the correct site of binding in the reduced enzyme? A conformational change is probably an essential part of the redox reactions and the coupling of electron to proton movements. Long-range conformational coupling is known in several helical haem proteins, for example cytochrome *c* and haemoglobin, but not in the β-sheet copper proteins[3].

The nature of the two different proton paths (see figure) is still uncertain. Protons move in very short hops (smaller than 1 Å) and require rotational movements of many donor and acceptor groups to make the process continuous. Moreover, in this enzyme protons move along unidirectional paths. Michel and co-workers[1] point with confidence to a path for the protons for the $O_2 \rightarrow 2H_2O$ conversion, which stops at haem $a_3$ and $Cu_B$ and starts from the inner side of the membrane (see Fig. 5*b* of ref. 1). These protons leave the $O_2$-binding site as water and hence need a large (water) channel to escape.

The problem path is the one that involves pumping of protons across the membrane (one way only)[3]. These proton movements must be tightly coupled to redox changes and gated. They must not reach the dioxygen reduction site because this would uncouple their obligatory separate path. The use of the $Cu_B$ histidine ligand as a swinging arm, as shown in Fig. 2 of ref. 1 and elsewhere, must surely fail in this respect. We have to conclude that the second proton-pumping path is still very uncertain and that we need several structures of different enzyme states to define this gated path, as Michel and colleagues[1] admit. It is salutary to remember the extensive work on different conformations that was necessary to follow protons in bacteriorhodopsin[6].

The new work shows once again that, in membranes, biologically engineered proton paths dominate bioenergetics at least equally with electron paths. These pathways, required in quinone reactions of photoreaction centres[7], in ATP synthetases[8] and now in cytochrome oxidase[1], are clearly also demanded by the new outline structures of particle I of mitochondria[9]. Many of the descriptions of energy transduction in textbooks will need rewriting. □

*R. J. P. Williams is in the Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QR, UK.*

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