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Strategy of research on the chemiosmotic mechanism of cytochrome oxidase

Peter Mitchell

Glynn Research Institute, Bodmin, Cornwall PL30 4AU, England

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In recent papers on protonmotive redox mechanisms in cytochrome oxidase in [(1987) FEBS Lett. 222, 235–245] and [Glynn Biological Research Reports (1987) 3, 1–7], I have suggested that a copper centre may enable the H_2O/OH or H_2O/O couple to act as the hydrogen-carrying arm of a redox loop by means of a $(CuOH_2)^+/(CuOH)^+$ or $(CuOH_2)^+/(CuO)^+$ system at the centre. I here explain that critical comments by Malmström [(1988) FEBS Lett. 231, 268–269] on the first of these papers, which might also be levelled at the second, depend on a misunderstanding. I also respond to Malmström's comment about testing conformationally coupled proton-pump mechanisms.

Chemiosmotic strategy; Redox loop concept; Cu center; Cu loop mechanism; Hydroxidemotive loop; Oxidemotive loop

In their book on cytochrome oxidase, Wikström and colleagues (on p.11 of [1]) stated that the "proton pumping function cannot be explained by the redox loop model simply because the oxidase contains only formal electron carriers." They concluded that "a fundamentally different kind of coupling mechanism was implicated, one that may generally be called a redox-linked proton pump...". As they subsequently remarked ([1], p.143), "the proton/electron coupling would necessarily be less 'direct'.. than in the redox loop case,..", and that view was generally accepted (see [2]). Referring correctly (*pace* Malmström [3]) to this usage of redox-linked pump in the context of cytochrome oxidase, my colleagues and I pointed out [4,5] that it was a mistake to conclude that redox loop mechanisms must necessarily be ruled out. Such, presumably, was the general preoccupation with the oxidase *enzyme*, that the possible hydrogen-carrying function of its *substrates* was overlooked, despite the precedent of the hydrogen-carrying role of the QH_2/Q substrate couple in cytochrome *c* reductase. Incidentally, it would not be surprising if some confusion had arisen over the definition of the term 'redox-linked proton pump', which we understand was intended to mean any kind of proton-translocating redox system. However, such problems of nomenclature, although worth our careful consideration, need not obscure the crucial issues of research strategy with which we are primarily concerned.

The key strategic question in cost-effective research is where to focus attention. I have adopted the classical (vectorial metabolic) chemiosmotic strategy, and have sought possible hydrogen-carrying couples, selected from the substrates and intermediates, that might function in a redox loop system in cytochrome oxidase, especially in connection with the chemistry of O_2 reduction at the binuclear centre. The observation that the redox poise of Cu_B in mitochondria is not shifted appropriately, relative to cytochrome *c*, when a large $\Delta\tilde{\mu}H^+$ is induced by the hydrolysis of ATP [6], is inconsistent with proposals that the proton pump is linked to electron transfer at Cu_A [7] or at haem *a* [1,8,9]. I think that Chance has been right to associate the protonmotive mechanism with events at the binuclear centre (see [10]).

Starting from a concept of metal-catalysed hydroxide-ion binding and translocation, I

Correspondence address: P. Mitchell, Glynn Research Institute, Bodmin, Cornwall PL30 4AU, England

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described the possible participation of a hydrogen-carrying H_2O/OH couple [in the form of a $(CuOH_2)^+/(CuOH)^+$ couple] at a copper centre [5]. I called the metal centre hydroxidemotive for local mechanistic reasons, and Malmström [3] objected that I had extended the meaning of redox loop. However, that objection was mistaken. As the reader may verify, the system that I described actually consisted of a protonmotive redox loop (carrying hydrogen one way and electrons the other) in series with an electron-conducting arm (see fig.2 of [5]). My more recent descriptions of so-called hydroxidemotive and oxidemotive Cu_B loop mechanisms [Glynn Biological Research Reports (1987) 3, 1–7, obtainable gratis from this Institute] likewise consist of hydrogen-conducting and electron-conducting redox loop arms, and involve no extension of the redox loop concept.

In my systematic development of the concept of chemicomotive ligand-conducting loops over the last 30 years (see [11,12]), I have been careful to recognise that a locally OH-conducting arm of a hydroxidemotive loop, or a locally O-conducting arm of an oxidemotive loop, actually produces a hydrogen-conducting arm, operating in the reverse direction, in the overall protonmotive loop system. This is because natural lipid membranes are permeable to H_2O, and the back-flow of H_2O converts the forward local conduction of 2 OH or O to an overall reverse flow of 2 H.

With respect to cost-effective research strategy, my view (nicely supported by Harold [2]) that redox loop mechanisms are generally relatively easy to test, because of their relative simplicity and chemical explicitness, does not seem to me to be belied by Malmström's comment that allosterically coupled systems are now becoming testable because of "the *extremely detailed* experimental information available.." (my italic).

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