Volume 222, number 2, 235–245 FEB 05115 October 1987

Review–Hypothesis

A new redox loop formality involving metal-catalysed hydroxide-ion translocation

A hypothetical Cu loop mechanism for cytochrome oxidase

Peter Mitchell

Glynn Research Institute, Bodmin PL30 4AU, England

Received 3 August 1987

A new hypothetical type of redox loop is described, which translocates hydroxide ions instead of protons. Conventional protonmotive redox loops use carriers of protons with electrons (e.g. QH_2/Q systems) to couple electron transfer to the translocation of protons. The putative hydroxidemotive redox loop uses carriers of hydroxide ions against electrons (e.g. transition-metal centres) to couple electron transfer to the translocation of hydroxide ions. This simple idea leads to the proposal of a hydroxidemotive Cu loop mechanism that may possibly be applicable to the Cu_A or Cu_B centre of cytochrome oxidase, and might thus account for the coupling of electron transfer to net proton translocation in that osmoenzyme.

Cytochrome oxidase; Cu center; Hydroxidemotive redox loop; Cu loop mechanism; Chemiosmotic coupling; Hydroxide-ion translocation

1. INTRODUCTION

It was persuasively argued by Wikström and colleagues (see e.g. [1], p.11) that the protonmotive action of cytochrome oxidase cannot be explained by a directly coupled redox loop type of formality, for the simple reason that the oxidase contains only formal electron carriers. The conclusion was widely drawn that proton pumping in cytochrome oxidase must occur by the indirectly coupled redox-linked type of mechanism described by Wikström and Krab [2,3], which generally uses such a broadly based concept of conformational energy transduction [1–6] that it is very difficult to test experimentally. However, there is good evidence that, in protonmotive QH_2 dehydrogenases containing only formal electron carriers, the quinone substrate functions as the trans-osmotic-barrier conductor of hydrogen according to Q loop and Q cycle mechanisms [7–9]. It follows that, in cytochrome oxidase, the substrate oxygen might likewise function as the conductor of hydrogen according to a protonmotive O loop or O cycle mechanism [10]; and these suggestions are being actively developed and explored [11]. However, efforts to establish the existence of the second oxygen-reducing centre, required by the O loop and O cycle mechanisms, have not so far yielded encouraging results. Consequently, I have thought it wise to continue to prospect for conceivable direct mechanisms of coupling between electron transfer and net proton translocation in cyto-

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

Abbreviations: Q, ubiquinone or plastoquinone; QH_2 ubiquinol or plastoquinol; Cu_A, Cu_B and Cu_X, electron-transferring copper centres in cytochrome oxidase; ENDOR, electron nuclear double resonance

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

chrome oxidase, giving a stoichiometry of one proton translocated per electron transferred as originally observed by Wikström (see [1,11–14]), in case a more promising mechanistic hypothesis might be found.

In the first part of this paper, I describe a new hypothetical class of redox loop designed to translocate hydroxide ions instead of protons. In the conventional protonmotive redox loop, electron transfer is coupled to proton translocation by a trans-osmotic-barrier system (such as a QH_2/Q system) that catalyses sym-coupled electron-proton transfer or hydrogen transfer [7,15]. In the hydroxidemotive redox loop, electron transfer is supposed to be coupled to hydroxide-ion translocation by a trans-osmotic-barrier system (such as a transition-metal centre) that catalyses anti-coupled electron/hydroxide-ion transfer, which corresponds to sym-coupled electron-hole–hydroxide-ion transfer or hydroxyl transfer. In a sym-coupled (or symfer) system, input of the one species (e.g. electron or electron-hole) induces input of the other (e.g. proton or hydroxide ion, respectively), whereas in an anti-coupled (or antifer) system, input of the one induces output of the other. The use of the electron-hole concept allows electron/hydroxide antifer to be conceived as electron-hole–hydroxide symfer.

The definitions of symfer and antifer [16] are broader than the definitions of symport and antiport. In symfer and antifer, the coupling of the motions of the two species does not necessarily require them to move along parallel pathways, and may require only one of the species (e.g. hydroxide ion) to pass through the osmotic barrier domain of the osmoenzyme [16].

These simple considerations lead to the proposal of a hypothetical hydroxidemotive Cu loop mechanism in which it is suggested that the Cu_A or Cu_B centre may catalyse anti-coupling between the translocation of hydroxide ions through the osmotic barrier domain in cytochrome oxidase and the transfer of electrons from cytochrome *c* to the haem group of the haem a_3-Cu_B binuclear centre (leaving open the question of the position of Cu_A relative to haem *a* and to another Cu centre [17,18], described here as Cu_X, that may participate in the process of electron transfer).

The general principle of the hydroxidemotive Cu loop mechanism was briefly outlined in the Croonian Lecture, delivered at the Royal Society, London, in May 1987 [19].

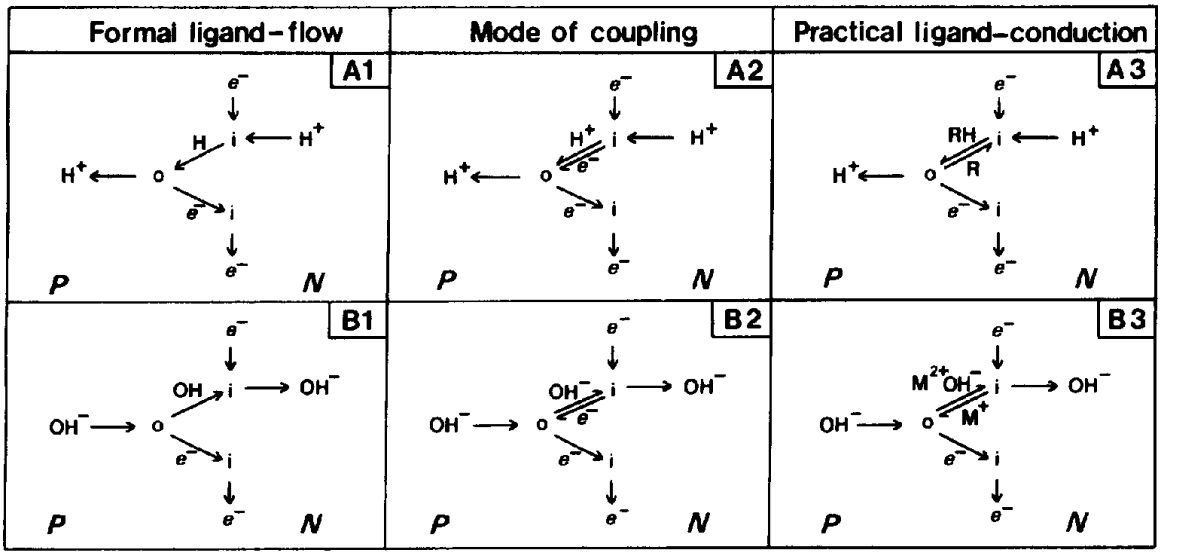

Fig.1. Conventional protonmotive redox loops (A) compared with hydroxidemotive redox loops (B). Further explanations are in the text.

2. HYDROXIDEMOTIVE REDOX LOOPS

2.1. *General conceptual principles*

The net effect of hydroxide-ion translocation is, of course, practically indistinguishable from that of proton translocation in a chemiosmotic system, because coupling membranes have a relatively high permeability to H_2O [20], and the deprotonation of H_2O is fast and reversible in aqueous media.

Fig.1 compares the conventional protonmotive redox loop (panels A) with the hydroxidemotive redox loop (panels B). The upper and lower links of each loop represent trans-osmotic-barrier pathways of ligand-specific conduction between centres of effective protonic input (i) and output (o) that equilibrate protonically with the aqueous media at low and high protonic potentials (*N* and *P*, respectively) on either side of the coupling membrane. Panels 1 show formal ligand flow. Panels 2 show the mode of electron:proton or electron:hydroxide-ion coupling (whether symport or antiport) in the upper link of each loop. Panels 3 indicate possible mechanisms of ligand conduction in the upper link of each loop, discussed in more detail below. The lower link of all the loops simply returns electrons through the osmotic barrier, so that there is no net (trans-osmotic-barrier) electron translocation in the loop formality, which is designed to represent the coupling of electron transfer to proton or hydroxide-ion translocation.

In the conventional A3 loop, R represents a group (such as a *p*-quinone oxygen) that carries a proton when in the electronated state (RH), and returns in the de-electronated state (R). In the hypothetical B3 loop, M represents a metal centre that carries a hydroxide ion when in the de-electronated state ($M^{2+}OH^-$, equivalent to M^+OH), and returns in the electronated state (M^+).

The formal analogies that can be traced between the conventional type of redox loop and the new hypothetical one suggest that the latter may be feasible in general principle. However, in the known protonmotive Q link systems, the QH_2/Q couple (corresponding to RH/R in the upper link of fig.1, A3) is highly mobile in the fluid hydrocar-

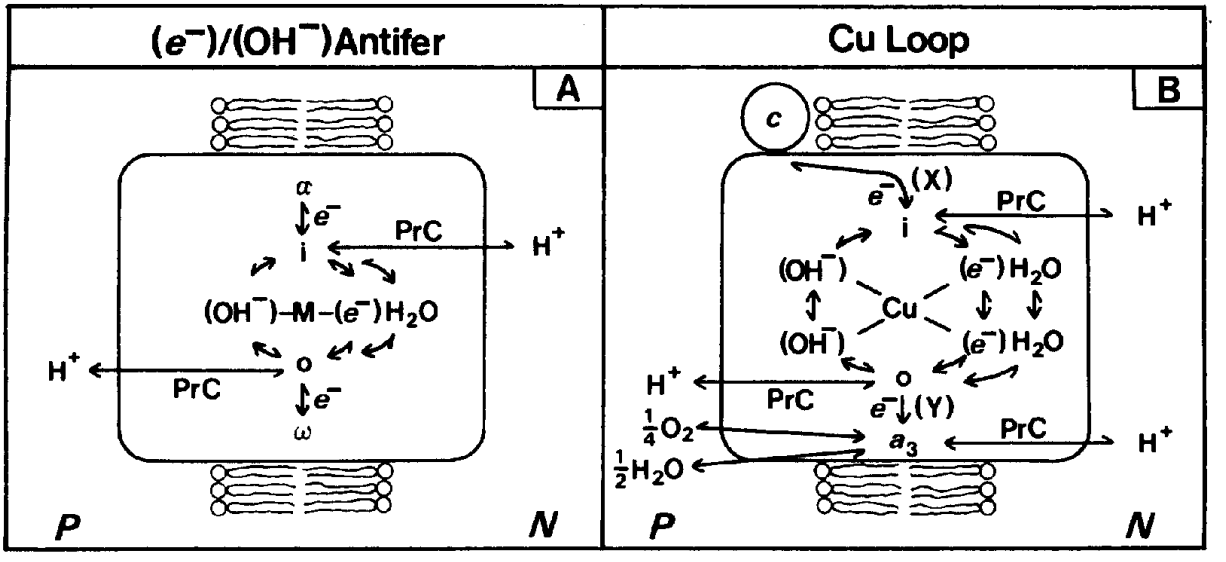

Fig.2. Ligand-conduction diagrams of hypothetical hydroxidemotive systems using transition-metal centres (M or Cu) to catalyse net proton translocation. Panel A shows a purely hypothetical Cu loop system. Panel B shows a corresponding Cu loop mechanism applied to the Cu_A or Cu_B centre of cytochrome oxidase, where X and Y represent the electron-conducting components leading to and from the protonmotive Cu centre. Note that the ligands shown on either side of the Cu atom are supposed to be bound, not simultaneously, but alternately during the cyclic process of hydroxide translocation. Further explanations are in the text.

bon osmotic barrier domain, and can readily move between the i and o sites through which the electrons drive the protons [9,11]. By contrast, the mobility of the metal centre M in the polypeptide osmotic barrier domain of a putative hydroxidemotive metalloprotein would presumably be relatively restricted; and to enable the centre to act as a mobile carrier [21], the bonds around the metal atom would have to be sufficiently mobile to facilitate the specific translocation of hydroxide ions through the centre between the o and i sites in concert with the transfer of electrons from donor to acceptor. Accordingly, I suggest that, although not impossible at an iron-porphyrin centre, such as haem *a*, the hydroxidemotive mechanism may be more readily applicable to a copper centre, such as Cu_A or Cu_B.

2.2. *Some corollaries of hydroxide-ion translocation*

The ligand-conduction diagram in fig.2A shows the upper link of the hypothetical hydroxidemotive system of panel B3 in fig.1, developed in more detail. In this diagram, the i and o centres are shown embedded in the osmotic barrier domain of an osmoenzyme polypeptide system, and they are assumed to be connected to their respective *N* and *P* aqueous domains by proton conductors (PrC) [15,16,22–25]. The electron/hydroxide-ion antifer (or electron-hole-hydroxide-ion symfer) is represented formally by the circulation of ligands around the metal centre M between i and o configurations, in which electrons are reversibly accepted from a donor site α and donated to an acceptor site ω. The mutually exclusive hydroxidated and electronated states of the metal centre are shown as (OH^-)-M and M-(e^-), respectively. In this type of system, it is evident that H_2O would have to be produced at the i centre and consumed at the o centre, and the H_2O would move in the opposite net direction to the hydroxide ion, and in the same net direction as the electronated state of the metal centre. Tight coupling between electron transfer and proton translocation would depend on the channelling of ligand conduction as represented in the diagrams and not generally otherwise [26]. For example, uncoupling would occur if a significant quantity of M-(e^-,OH^-) were produced and were mobile between the i and o configurations of M.

3. POSSIBLE MECHANISMS OF HYDROXIDEMOTIVE Cu LOOPS

3.1. *General ligand-conduction diagrams*

The ligand-conduction diagram in fig.2B shows the $(e^-)/(OH^-)$ antifer system of diagram A applied to cytochrome oxidase, with the hydroxidemotive function of M attributed to Cu_A or Cu_B. This diagram is meant to describe the required topological connectedness of the components, catalysing electron transfer from cytochrome *c* to the binuclear O_2-reducing centre a_3-Cu_B, and facilitating proton transfer between the *P* and *N* aqueous media and the appropriate o and i centres. But, this diagram is not meant to show the actual positions of the centres and conducting pathways, or the shape of the cytochrome oxidase molecule.

3.2. *Conceivable Cu loop molecular mechanisms*

The description of local hydroxide-ion translocation and net proton translocation by the putative Cu loop system is largely formal in fig.2B. Nevertheless, this diagram is instructive with respect to some of the general principles governing the possible development of more detailed molecular mechanisms that might be applicable to the Cu_A or Cu_B centre of cytochrome oxidase. In view of the major uncertainties concerning the Cu centres, summarised below, it would be premature to attempt to provide a specific detailed mechanistic diagram of the possible Cu loop system in the present paper. But I will develop, somewhat further, the set of general principles by means of which one may explore some of the possibilities.

Much of the experimental evidence concerning the geometry and ligation at the Cu centres in cytochrome oxidase has been discussed by Malmström [27,28], Chan and colleagues [29–33], and Scott and co-workers [34,35]. Since the Cu_B centre is normally EPR silent, and the contributions by Cu_A and Cu_B to the X-ray signals given by the oxidase cannot be unequivocally distinguished in the native oxidase [33,35], it was inferred that all three of the ligands at Cu_B may be N or O, although the possibility of one Cu-S interaction at Cu_B could not be completely ruled out [35]. From the anomalous EPR signal of Cu_A [36–38], taken in conjunction with ENDOR [31] and X-ray absorption studies [34,35,39], it was inferred that two S and one N may be ligands at Cu_A.

New uncertainties about the Cu_A ligand system were recently introduced by observations of Buse and colleagues [17] and Kadenbach et al. [18], which imply that cytochrome oxidase from most sources may be a three-copper two-haem *a* protein. The careful work of Buse's group, using inductively coupled plasma atomic emission spectroscopy, indicated that *Paracoccus denitrificans* and ox heart oxidases, which contain subunits I and II, have 3 copper atoms per molecule, whereas *Thermus thermophilus* oxidase, in which subunit II is replaced by a cytochrome c_1 subunit, has only 2 copper atoms per molecule [17]. They suggested that, in cytochrome oxidase from most sources, subunit I may contain the generally accepted centres, haem *a*, Cu_A, haem a_3 and Cu_B, and that subunit II may contain an additional copper centre (described here as Cu_X). The conclusion of Buse's group that the ratio of Cu to Fe is 3:2 in cytochrome oxidases from most sources was confirmed by Kadenbach's group, using a proton-induced X-ray emission method on cytochrome oxidases isolated from various mammalian species and tissues [18].

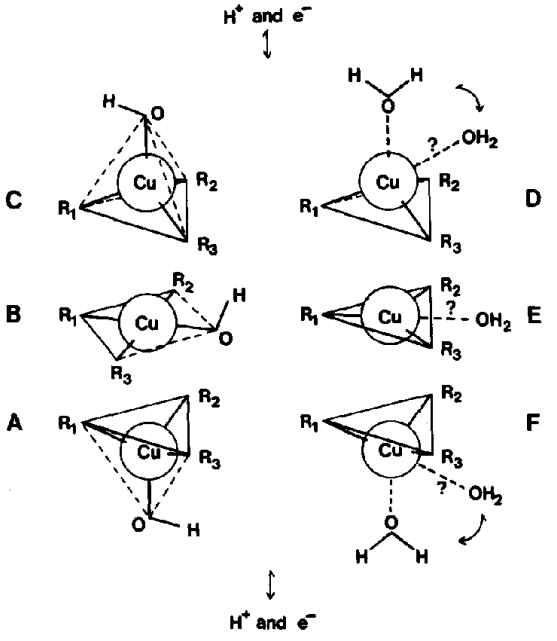

Fig.3. Suggested type of mechanism for coupling electron transfer to hydroxide translocation at the Cu_A or Cu_B centre of cytochrome oxidase. R_1, R_2 and R_3 represent amino acid ligands in the osmotic barrier domain of the oxidase, the i site being above the plane of these ligands, and the o site being below it. Diagrams A–F represent the sequence of states in the working configurational cycle. In passing back from the i to the o site at the Cu centre, the H_2O may or may not travel in close proximity to the copper atom, as indicated by the question marks in diagrams D, E and F. The circle round Cu does not represent the size of the Cu atom. Further explanations are in the text.

In summary, on the basis that cytochrome oxidase contained only 2 copper centres, Cu_A and Cu_B, the experimental evidence was interpreted to show that the ligation of Cu_A involved 1 or 2 histidine nitrogens, possibly 1 oxygen, and 1 or 2 cysteine sulphurs [33,35]. But, if the existence of Cu_X [17,18] were to be confirmed, and if, as seems just possible, Cu_X is ligated to cysteine sulphurs in subunit II [40–42], the ligation of both Cu_A and Cu_B in subunit I might involve only nitrogen and oxygen, like the type 2 copper in laccase [43–45]. This could be important in the context of the proposed hydroxidemotive redox loop mechanism, because hydroxide ligands to Cu are known to be favoured by soft ligands such as N, but not by hard ligands such as S. For that reason also, one might consider Cu_B to be the more likely centre for the hydroxidemotive mechanism.

Incidentally, the evidence from proton and ^{17}O magnetic resonance relaxation studies of the oxidised type 2 copper centre in laccase by Pecht and colleagues [45] has shown that it is possible for a copper centre to show very slow exchange of hydroxide ions and water with the aqueous media, and yet for hydroxide, bound at the centre, to be readily accessible to protons, presumably through one or more specifically proton-conducting pathways. This is particularly noteworthy, because copper generally exhibits very fast ligand exchange, and practically the only way for the rapidly exchanging hydroxide ligand to be prevented from equilibrating with the aqueous media would be by enclosure of the copper ligand system in a non-polar domain impermeable to hydroxide ions and water, as suggested by Pecht et al. [45].

3.3. *Possible mechanisms of hydroxide-ion translocation around the copper atom at the Cu_A centre*

Comparisons of the Cu_A centre in cytochrome oxidase with the metal centres in other copper proteins, notably laccase, azurin and plastocyanin,

have suggested that the orbital containing the unpaired electron at the Cu_A centre in the oxidised state may be a copper 3d orbital hybridised with copper 4p and 4s orbitals, and that the coordination shell at Cu_A may be distorted from a square planar to a near tetrahedral configuration by the ligand positions in the surrounding polypeptide system [27,28,31,33]. This is obviously an interesting suggestion from our point of view, because it offers a possible mechanism for the mobility that is presumably required to enable the hydroxide ion to be conducted around the Cu centre in concert with the redox cycle. Chan and colleagues recently invoked a cyclic change at the Cu_A centre, between a distorted tetrahedral configuration in the oxidised state, and a planar trigonal configuration in the reduced state, in a mechanism proposed for conducting protons across the Cu atom between a tyrosine phenolate ligand on one side and a thiolate ligand on the other [33]. In that proposal the proton was not, of course, considered to be bonded to the Cu atom, and the chemical and physical facilities required to transport the proton past the Cu atom were not as strictly defined as those that may be available to enable a hydroxide ion bonded to the Cu atom to pass around it from one side to the other, as proposed here.

Assuming a coordination number of 4, and using the valence bond formality of Pauling, the translocation of the hydroxide ion around the copper atom at the oxidised Cu_A or Cu_B centre could be facilitated by hybridisation between the copper 3d orbital and the copper 4p and 4s orbitals. If, as illustrated in fig.3, the Cu centre were bound by three amino acid ligands R_1, R_2, and R_3 in the hydrophobic osmotic barrier domain of the polypeptide system of the oxidase, with centres i and o respectively above and below the plane of these ligands (cf. fig.2B), rotational motion and inversion of the hydroxide ligand might presumably occur by transition of the copper ligand geometry from a tetrahedral sp^3 configuration (fig.3, state A), with the hydroxide ligand on the (downward) o side, through a square planar dsp^2 configuration (state B), with the hydroxide ligand in the plane of the other three ligands, and on to an inverted tetrahedral sp^3 configuration (state C), with the hydroxide ligand on the (upward) i side. The configurational cycle would be completed by electronation and protonation (state D), movement of the copper atom back through the plane of the amino acid ligands (state E), and de-electronation and deprotonation (state F). The assumed reversible lengthening of the Cu–O bond, and possible dissociation of the water ligand, at D and F, are based on the principle that reduction generally causes loss of coordination in metal complexes. During the configurational cycle, making reasonable assumptions about the bond lengths and angles of the copper ligands, the oxygen atom of the hydroxide ligand, would move about 5 Å, or possibly more, through the plane of the three amino acid ligands in the osmotic barrier domain, assumed to intervene between the i and o centres; and the copper atom would also move a distance approaching 2 Å.

If the coordination number of the copper atom at the oxidised Cu centre is 5 or 6, rather than 4, the mechanism of hydroxide translocation could be essentially similar to that suggested in fig.3, the general principle of which corresponds to a Berry pseudorotation mechanism (see [46], pp.247–249), originally applied to the interchange of axial and equatorial ligands. In such mechanisms a given ligand may swing only partially around the centre to which it is bonded, and that could apply to the process of hydroxide translocation at the Cu_A centre. I must therefore emphasise that the diagrams of fig.3 should be taken only to illustrate the general principle of hydroxide translocation around the Cu centre.

At all events, the proposition that the hydroxide ion (and perhaps the H_2O molecule) might swing around the copper atom, as indicated in fig.2, appears to be more realistic, and less purely formal, than one might have expected.

3.4. *Possible mechanisms of water translocation from one side of the Cu centre to the other*

The translocation of water from the i to the o site, shown in fig.2B, would not necessarily require the H_2O molecules to swing around the copper atom in close proximity to it (by contrast with hydroxide translocation), but H_2O translocation might possibly occur by equilibration of H_2O (but not of OH^-) at the i and o sites with more remote domains on either side of the osmotic barrier domain in which the Cu centre is supposed to be situated.

3.5. *Coupling between electron transfer and hydroxide translocation in the Cu loop mechanism*

The ligand-conduction diagrams of the Cu loop mechanism in fig.2B can conveniently be considered to describe continuous sets of states with corresponding configurations through which the system passes in a catalytic configurational cycle. We can readily recognise four main configurations (as indicated in fig.2B), and four transitional configurations. There is one reduced and one oxidised configuration in which the hydroxide ligand and water may equilibrate protonically via the proton conductor (PrC) connected to the i site, and one reduced and one oxidised configuration in which the hydroxide ligand and water may equilibrate protonically via the proton conductor connected to the o site. The four transitional configurations correspond to the transitions between neighbouring pairs of these main configurations. Two of the transitions correspond to translocations and two correspond to electron transfer and corresponding hydroxide antifer. The efficient operation of the net protonmotive action of the oxidase indicates that each of the transitions between neighbouring states might be freely reversible with respect to the Cu centre, even though the acceptance and transfer of electrons through the oxygen-reducing a_3-Cu_B centre is known to be only partially reversible [47,48].

Electron transfer could be most simply coupled to hydroxide-ion translocation (fig.2B) if the i to o transition of the electronated Cu centre could not occur without dehydroxidation at the i site, the o to i transition could not occur without hydroxidation at the o site, and if electronic equilibration, either with the electron donor or with the electron acceptor, were restricted to the i and o configurations, respectively. In that case protonation of the hydroxide ligand and its conversion to H_2O at the i site would be linked to electron acceptance from cytochrome *c* and deprotonation of H_2O and its conversion to hydroxide at the o site would be linked to electron donation to the haem group of the haem a_3-Cu_B centre. The required specificity of the translocation of (HO^-)-Cu and Cu-(e^-) or Cu-(e^-)H_2O around the copper atom (and thus, through the osmotic barrier) would be ensured by the virtual non-existence of -Cu and Cu-(e^-,OH^-). Thus, the conditions that would have to be met by the Cu centre in a feasible well-coupled Cu loop mechanism might be relatively straightforward. The electrical repulsion between the electron and the hydroxide ion, and the expected increase in the copper coordination number by oxidation, would tend to work in favour of the appropriate deprotonation and protonation of H_2O and OH^- at the o and i sites, respectively, during the redox cycle; and tight channelling of the catalytic configurational cycle could be strongly assisted by electrostatic energy minimisation in the presumably low-dielectric-strength environment of the Cu centre.

The requirement that electronic contact between the Cu centre and the electron donor and acceptor systems should be established only in the appropriate i and o configurations would presumably depend on the position of the copper atom, of the components of the participating H_2O/OH^- couple, and on other related configurational circumstances requiring a more detailed description of the Cu centre than it is possible to give at present. But I suggest that the required configuration-dependent ligand conduction might be partly dependent on the involvement of histidine nitrogen, cysteine sulphur, and/or other ligands at the Cu centre, both in electron transfer and in the reversible protolytic actions assumed to be associated with hydroxide binding and translocation.

3.6. *Midpoint reduction potential pH dependence*

In the Cu loop mechanism of fig.2B, the midpoint reduction potential of the Cu_A centre might decrease with increasing pH, because electronation would be coupled to net protonation. However, in an equilibrium redox titration of the oxidase, the reduction of the oxidised Cu centre would be accompanied by net protonation (and the midpoint reduction potential would be pH dependent) only inasmuch as the oxidised and reduced populations of the centre were dominated by the species shown in fig.2B. In other words, the midpoint reduction potentials would be pH independent if oxidised and reduced configurations that were equally protonated were the predominant species in equilibrium redox titrations. This would correspond to cases in which the translocational state transitions were relatively fast, so that the appropriate equally protonated states could ac-

cumulate without failure of the kinetic competence of the system. Analogous relationships were considered in [26], and have been discussed by Chan et al. [49]. The pH dependence or independence of the midpoint reduction potentials could, of course, also be affected by linked-function redox-dependent protolytic actions, manifested at sites in the Cu centre (perhaps involving amino acid ligands), or at peripheral sites in the protein, and not shown in fig.2.

As indicated above (and see [49]), the widely held view that redox couples involved in net proton translocation must have pH-dependent midpoint reduction potentials is mistaken. Experimental studies, in which the midpoint reduction potentials of Cu_A and Cu_B have been found to be only slightly affected by pH in the range 6.0–8.5 [50–52], are not, therefore, inconsistent with the Cu loop mechanism suggested in this paper.

3.7. *The driving force on the catalytic configurational cycle in the Cu loop mechanism*

In the Cu loop mechanism, the driving force on the catalytic configurational cycle would be given by the total free-energy decrease of the Cu_A centre system, following electronation and de-electronation. Thus, if they were to occur with equal probability in the catalytic cycle, the configurations of the Cu centre, following electron acceptance from the donor system and electron donation to the acceptor system, would represent states of increasing stability. The electrostatic energy of the Cu centre would be expected to play a major part in driving the catalytic cycle. But changes of stress in the coordination sphere of the copper atom at the Cu centre, associated with electron transfer and changes of geometry during the ligand rearrangements associated with hydroxide-ion translocation, might also be expected to contribute significantly to the driving and energetic stabilisation of the set of states that make up the catalytic configurational cycle, and enable electron transfer to be tightly and reversibly coupled to net proton translocation.

4. DISCUSSION

In previous schemes designed to explain the net protonmotive function of cytochrome oxidase, attention has been focused on the proton as the chemical species supposed to be primarily translocated by the action of electron transfer [1–6,29,33,49]. For example, in the original proton-pump mechanism proposed by Chan and colleagues, electronation and de-electronation of the Cu_A centre were supposed to drive the entry and exit of protons via the protonation and deprotonation of one of the sulphur ligands [29]. In a new and more detailed proton-pump model, Chan and co-workers [33,49] proposed that the protons are translocated across the Cu_A centre by a redox-driven ligand-substitution mechanism, with associated bond-orientational changes, involving the alternate protonation and Cu ligation of a cysteine thiolate ligand and a tyrosine phenolate ligand on opposite sides of the Cu_A centre. In their view, "the directionality of the coupled electron and proton flows in cytochrome *c* oxidase makes the mechanism of proton translocation by this enzyme fundamentally different from the 'redox loop' mechanism..." [49]. For application to cytochrome oxidase, they developed a general kinetic protonmotive rationale, including leak pathways [49], which was much the same in general principle as a multi-channel ligand-conduction rationale used to describe the tightness of coupling in symport and antiport processes ([26] and see [15,16]). But it was evidently difficult to apply this rationale strictly to their new proton-pump model [33], since, as in their earlier model [29], they did not fully explain how electron transfer would drive the protons vectorially through the proposed proton-carrying ligand system.

My main object in the present paper has been to consider the possibility that the net protonmotive function of cytochrome oxidase might be more easily and explicitly described if we were to focus attention on the hydroxide ion, instead of the proton, as the chemical species that may be primarily translocated by the action of electron transfer. As indicated in the first part of this paper, this idea arises from general redox-loop considerations, which suggest that transition-metal centres, such as the Cu_A or Cu_B centre of cytochrome oxidase, would be better adapted chemically to translocate hydroxide ions than to translocate protons.

As shown in fig.2, the enclosure of the metal centre in the osmotic barrier domain of an os-

moenzyme polypeptide system, with the i and o sites connected to their respective *N* and *P* aqueous domains by proton conductors, would give rise to the participation of H_2O as proton carrier in the type of net protonmotive system postulated here, which depends on the primary translocation of hydroxide ions. This is an interesting aspect of my proposals for two reasons. First, the proposed participation of the protons at the i and o sites of the putative hydroxidemotive metal centre allows some mechanistic subtleties that would not otherwise be available. Second, the return movement of the water that would be induced by the primary translocation of hydroxide ions either might be confined to the locality of the transition-metal centre catalysing hydroxide translocation, or might occur generally through the hydrocarbon domain of the coupling membrane, which is known to be fairly permeable to water [20]. If the latter were the case, the role of H_2O as a distributed carrier of protons (with hydroxide ions) through the membrane hydrocarbon in the putative hydroxidemotive function of cytochrome oxidase would be somewhat analogous to the role of ubiquinol and plastoquinol as distributed carriers of protons (with electrons) through the membrane hydrocarbon in the protonmotive function of cytochrome *c* and plastocyanin reductases.

The anion-carrying properties of some artificial organometallic and organic cationic compounds may be relevant to the general concept of the hydroxidemotive redox loop. Triethyltin and other organotin compounds, and diphenyleneiodonium and derivatives, have been shown to catalyse fairly tightly coupled hydroxide/chloride antiport through lipid membranes [53–57]. More interestingly, the benzyl viologen cation radical (BV^+) is a good carrier of nitrate through lipid membranes, whereas oxidised benzyl viologen (BV^{2+}) is not [58]; and it has been suggested that benzyl viologen may function both as an electron carrier and as an ionophore while catalysing the dithionite:nitrate oxidoreductase reaction in *P. denitrificans* [58]. However, the carriers involved in these artificial reactions are known to be relatively mobile in the hydrocarbon domain of the membrane. Thus, although they indicate the general feasibility of hydroxide translocation by cationic centres, they are not relevant in the context of the very specific mobility and accessibility postulated for the Cu loop mechanism of hydroxide translocation in cytochrome oxidase.

It is noteworthy that in laccase, one of the ligands of the type 2 copper centre appears to be hydroxide or water, and the presence of the hydroxide ligand at the centre in the oxidised state may inhibit electronation of the centre under certain conditions [27,43–45]. These interesting observations suggest that electronation of the type 2 copper centre may be coupled to protonation of the hydroxide ligand, and that the alternative binding of OH^- or H_2O may be associated with a local protolytic action [45], as postulated here for the Cu_A or Cu_B centre of cytochrome oxidase.

The Cu loop mechanism, although inevitably speculative in the present state of knowledge, is nevertheless useful because it suggests new experiments. The proposed participation of hydroxide as a ligand at the Cu_A or Cu_B centre of cytochrome oxidase may be susceptible to experimental investigation by electromagnetic methods; and more detailed structural information about the oxidase would obviously help to bring experimental criticism to bear on this type of model.

Like earlier suggestions that the Cu_A centre couples electron transfer to net proton translocation [29,33], the Cu loop mechanism involving either Cu_A or Cu_B implies that the application of a protonmotive potential difference across the membrane, positive on the *P* side, should reverse electron transfer between cytochrome *c* and haem a_3 in the presence of oxygen. Hinkle and colleagues [59] observed that, in aerobic suspensions of liposomes inlaid with cytochrome oxidase, a steady-state protonic potential difference, positive on the *P* side, induced reduction of haem *a* relative to haem a_3, but not relative to cytochrome *c*. This would be compatible with a net proton-translocating function of Cu_A, haem *a*, or Cu_B. It should be possible to determine which of these centres was protonmotive by determining the effects of independently varying the protonic potentials of the *P* and *N* aqueous media on the redox poises of Cu_A, Cu_B and haem *a*, in cytochrome oxidase liposomes, or in other cytochrome oxidase preparations designed to study the partial redox and protolytic actions characteristic of the o and i aspects of the oxidase.

I have not included Cu_X as a possible candidate for the net protonmotive function of the oxidase in

this discussion, because the oxidase of *T. thermophilus* lacks Cu_X but nevertheless exhibits normal protonmotive activity [60].

5. CONCLUSIONS

There are two crucial requirements for tight coupling of net proton translocation to electron transfer in the putative Cu loop mechanism. The transition between the i and o configurations of the H_2O/OH^- ligand system at the Cu centre would have to correspond to H_2O/OH^- antiport (or H^+ uniport) through the osmotic barrier. Electron exchange with the normal donor and acceptor systems would have to be open alternately and exclusively when the H_2O/OH^- ligand system at the Cu centre is in the appropriate alternative i and o proton-exchange configurations.

If the Cu_A or Cu_B centre does not have a net proton-translocating function in cytochrome oxidase, or if it does have such a function but does not operate by a hydroxidemotive redox loop type of mechanism, it should be fairly straightforward to design experiments capable of revealing those facts. Meanwhile, the knowledge and ideas reviewed in this paper suggest that the mechanism of net proton translocation by cytochrome oxidase may not necessarily be fundamentally different from the 'redox loop' mechanism.

The conceptual invention of the hydroxidemotive redox loop may turn out to have been a fruitful theoretical departure in a broader context than that of cytochrome oxidase action.

ACKNOWLEDGEMENTS

I thank Gebhard von Jagow, Roy Mitchell, John Moody, David Moss, Peter Nicholls, Peter Rich and Ian West for discussions and criticisms that helped to prompt and define the conjectures considered in this paper, Willem Koppenol, Robin Perutz, Andrew Thomson and Michael Wilson for advice over specific points of fact and nomenclature, Sunney Chan and Marten Wikström for sending me preprints of [33] and [48] respectively, and Ann Severn and Robert Harper for assistance in preparing the manuscript and figures. Financial support was provided by payments under contract no. ST2P-0056-1-UK (CD) of the CEC Stimulation Action Programme, and by benefactors named in the brochure of the UK Registered Charity called The Glynn Research Foundation Ltd.

REFERENCES

[1] Wikström, M., Krab, K. and Saraste, M. (1981) Cytochrome Oxidase: A Synthesis, Academic Press, London.
[2] Wikström, M. and Krab, K. (1979) Biochim. Biophys. Acta 549, 177–222.
[3] Wikström, M. and Krab, K. (1980) Curr. Top. Bioenerg. 10, 51–101.
[4] Azzi, A., Bill, K., Bolli, R., Casey, R.P., Nałęcz, A. and O'Shea, P. (1985) in: Structure and Properties of Cell Membranes, vol.II (Benga, G. ed.) pp.105–137, CRC Press, Boca Raton, FL.
[5] Chance, B. and Powers, L. (1985) Curr. Top. Bioenerg. 14, 1–19.
[6] Malmström, B.G. (1985) Biochim. Biophys. Acta 811, 1–12.
[7] Mitchell, P. (1965) Biol. Rev. 41, 445–502.
[8] Mitchell, P. (1976) J. Theor. Biol. 62, 327–367.
[9] Lenaz, G. ed. (1985) Coenzyme Q, Wiley, Chichester.
[10] Mitchell, P., Mitchell, R., Moody, A.J., West, I.C., Baum, H. and Wrigglesworth, J.M. (1985) FEBS Lett. 188, 1–7.
[11] Mitchell, P. (1987) in: Advances in Membrane Biochemistry and Biophysics (Kim, C. et al. eds) Plenum, New York, in press.
[12] Mitchell, R., West, I.C., Moody, A.J. and Mitchell, P. (1986) Biochim. Biophys. Acta 849, 229–235.
[13] West, I.C. (1986) Biochim. Biophys. Acta 849, 236–243.
[14] West, I.C., Mitchell, R., Moody, A.J. and Mitchell, P. (1986) Biochem. J. 236, 15–21.
[15] Mitchell, P. (1981) in: Of Oxygen, Fuels, and Living Matter, pt 1 (Semenza, G. ed.) pp.1–160, Wiley, Chichester.
[16] Mitchell, P. (1977) Symp. Soc. Gen. Microbiol. 27, 383–423.
[17] Steffens, C.M., Biewald, R. and Buse, G. (1987) Eur. J. Biochem. 164, 295–300.
[18] Bombelka, E., Richter, F.-W., Stroh, A. and Kadenbach, B. (1986) Biochem. Biophys. Res. Commun. 140, 1007–1014.
[19] Mitchell, P. (1988) Proc. Roy. Soc. B, Croonian Lecture 1987, in press.
[20] Lipschitz-Farber, C. and Degani, H. (1980) Biochim. Biophys. Acta 600, 291–300.

[21] Mitchell, P. (1987) in: Integration and Control of Metabolic Processes: Pure and Applied Aspects (Kon, O.L. et al. eds) pp. 231–245, Cambridge University Press and ICSU Press, Cambridge and Miami.
[22] Mitchell, P. (1969) Exp. Theor. Biophys. 2, 159–216.
[23] Glasser, L. (1975) Chem. Rev. 75, 21–65.
[24] Nagle, J.F. and Tristram-Nagle, S. (1983) J. Membrane Biol. 74, 1–14.
[25] Senior, A.E. (1983) Biochim. Biophys. Acta 726, 81–95.
[26] Mitchell, P. (1967) Adv. Enzymol. 29, 33–87.
[27] Malmström, B.G. (1982) Annu. Rev. Biochem. 51, 21–59.
[28] Malmström, B.G. (1983) in: Biological Oxidations (Sund, H. and Ullrich, V. eds) pp.189–200, Springer, Berlin.
[29] Chan, S.I., Bocian, D.F., Brudvig, G.W., Morse, R.H. and Stevens, T.H. (1979) in: Cytochrome Oxidase (King, T.E. et al. eds) pp.177–188, Elsevier/North-Holland, Amsterdam, New York.
[30] Brudvig, G.W., Bocian, D.F., Gamble, R.C. and Chan, S.I. (1980) Biochim. Biophys. Acta 624, 78–89.
[31] Stevens, T.H., Martin, C.T., Wang, H., Brudvig, G.W., Scholes, C.P. and Chan, S.I. (1982) J. Biol. Chem. 257, 12106–12113.
[32] Blair, D.F., Martin, C.T., Gelles, J., Wang, H., Brudvig, G.W., Stevens, T.H. and Chan, S.I. (1983) Chem. Scr. 21, 43–53.
[33] Gelles, J., Blair, D.F. and Chan, S.I. (1987) Biochim. Biophys. Acta, in press.
[34] Scott, R.A., Cramer, S.P., Shaw, R.W., Beinert, H. and Gray, H.B. (1981) Proc. Natl. Acad. Sci. USA 78, 664–776.
[35] Scott, R.A., Schwartz, J.R. and Cramer, S.P. (1986) Biochemistry 25, 5546–5555.
[36] Malmström, B.G. (1973) Q. Rev. Biophys. 6, 389–431.
[37] Aasa, R., Albracht, S.P.J., Falk, K.-E., Lanne, B. and Vänngård, T. (1976) Biochim. Biophys. Acta 422, 260–272.
[38] Peisach, J. and Blumberg, W.E. (1974) Arch. Biochem. Biophys. 165, 691–708.
[39] Li, P.M., Gelles, J., Chan, S.I., Sullivan, R.J. and Scott, R.A. (1987) Biochemistry 26, 2091–2095.
[40] Steffens, G.J. and Buse, G. (1979) in: Cytochrome Oxidase (King, T.E. et al. eds) pp.79–90, Elsevier/North-Holland, Amsterdam, New York.
[41] Winter, D.B., Bruyninckx, W.J., Foulke, F.G., Grinich, N.P. and Mason, H.S. (1980) J. Biol. Chem. 255, 11408–11414.
[42] Darley-Usmar, V., Capaldi, R. and Wilson, M.T. (1981) Biochem. Biophys. Res. Commun. 103, 1223–1230.
[43] Andréasson, L.-E. and Reinhammar, B. (1976) Biochim. Biophys. Acta 445, 579–597.
[44] Andréasson, L.-E. and Reinhammar, B. (1979) Biochim. Biophys. Acta 568, 145–156.
[45] Goldberg, M., Vuk-Pavlovic, S. and Pecht, I. (1980) Biochemistry 19, 5181–5189.
[46] Huheey, J.E. (1983) Inorganic Chemistry: Principles of Structure and Reactivity, 3rd edn, Harper & Row, New York.
[47] Wikström, M. (1981) Proc. Natl. Acad. Sci. USA 78, 4051–4054.
[48] Wikström, M. (1987) Chem. Scr., in press.
[49] Blair, D.F., Gelles, J. and Chan, S.I. (1986) Biophys. J. 50, 713–733.
[50] Van Gelder, B.F., Van Rijn, J.L.M.L., Schilder, G.J.A. and Wilms, J. (1977) in: Structure and Function of Energy-Transducing Membranes (Van Dam, K. and Van Gelder, B.F. eds) pp.61–68, Elsevier/North-Holland, Amsterdam, New York.
[51] Wilson, D.F., Erecinska, M. and Owen, C.S. (1976) Arch. Biochem. Biophys. 175, 160–172.
[52] Lindsay, J.G., Owen, C.S. and Wilson, D.F. (1975) Arch. Biochem. Biophys. 169, 492–505.
[53] Selwyn, M.J., Dawson, A.P., Stockdale, M. and Gains, N. (1970) Eur. J. Biochem. 14, 120–126.
[54] Dawson, A.P. and Selwyn, M.J. (1974) Biochem. J. 138, 349–357.
[55] Watling-Payne, A.S. and Selwyn, M.J. (1974) Biochem. J. 142, 65–74.
[56] Gatley, S.J. and Sherratt, H.S.A. (1976) Biochem. J. 158, 305–315.
[57] Gatley, S.J. and Sherratt, H.S.A. (1976) Biochem. J. 158, 317–326.
[58] Kim, C.-H., Kristjansson, K., White, M.M. and Hollocher, T.C. (1982) Biochem. Biophys. Res. Commun. 108, 1126–1130.
[59] Moroney, P.McG., Scholes, T.A. and Hinkle, P.C. (1984) Biochemistry 23, 4991–4997.
[60] Sone, N., Yanagita, Y., Hon-Nami, K., Fukumori, Y. and Yamanaka, T. (1983) FEBS Lett. 155, 150–154.