Pure & Appl. Chem., Vol. 59, No. 6, pp. 749–758, 1987.
Printed in Great Britain.

Cytochrome oxidase: a perspective

Graham Palmer

Department of Biochemistry, Rice University, Houston, TX, U.S.A.

Abstract. Cytochrome oxidase from beef heart mitochondria is a 204 kDa large protein consisting of 13 subunits of which the numbers I-III are encoded by the mitochondrial DNA, while the remaining subunits (IV-VIII) are encoded by the nuclear genome. The protein contains 2 iron heme groups (cytochrome a and cytochrome a_3), 2 Cu centres (Cu_A and Cu_B) and 1 Zn atom. The heme groups and the Cu_B centre are located in subunit I, the Cu_A centre is located on subunit II, whereas subunit Vb contains the Zn site. The spectroscopic features of the oxidase are reviewed in relation to the structure of the metal centres. Cytochrome a occurs in the form of a low-spin bis-imidazole centre; Cu_A exhibits a high degree of covalency through which a large part of the unpaired electron is delocalized over 1 (or possibly even 2) sulfur ligands. Cytochrome a_3 and the Cu_B site form an antiferromagnetically, tightly coupled dinuclear centre, located at the dioxygen reducing side of the protein. The mechanism of electron transfer and the possible proton pumping activity of the enzyme are reviewed in the light of the available structural evidence.

INTRODUCTION

The inner membrane of the mitochondrion contains three electron-transferring enzyme complexes which play crucial roles in the conservation of biological free energy. These enzyme complexes are the NADH-ubiquinone oxidoreductase (complex I), the ubiquinol-ferricytochrome c oxidoreductase (complex III) and the ferrocytochrome c-dioxygen oxidoreductase (cytochrome oxidase, complex IV) (Fig. 1). The transfer of electrons from donor to acceptor catalyzed by these complexes leads to the creation (and maintenance) of a proton gradient across the inner membrane with the inner space (the matrix) becoming alkaline relative to the exterior. This differential in pH is created as a result of two processes: (i) a consumption of protons in the matrix as a consequence of the chemistry of the reaction. These so-called "scalar" protons are typified by the protons utilized during the reduction of oxygen to water by cytochrome oxidase; (ii) A net transport of protons from the matrix to the exterior driven by some of the free energy released during the electron transport process(es). In this latter case the enzyme is said to exhibit a "pump" activity. The transport of these "vectorial" protons is not necessarily a direct consequence of the transfer of electrons between the participating enzyme-bound redox centers but may be a result of some indirect, longer-range process. Subsequently the free energy stored in the proton electro-osmotic gradient drives the ATP synthetase (complex V) to produce ATP.

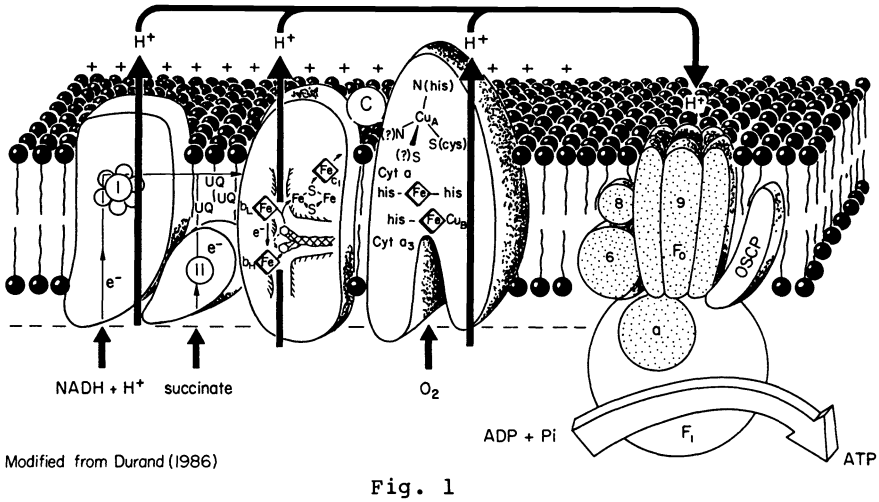


Fig. 1

By far and away the most intensively studied of these electron transfer complexes is cytochrome oxidase, particularly the protein isolated from the mitochondria of beef hearts. This is a large and complicated molecule. Its

Table 1: Comparison of subunits of bovine and yeast cytochrome oxidase.

YEAST	MOL MASS (kDa)	HEART[1]	EXPOSURE[2]	COMMENTS
	Mitochondrial Subunits			
I	57	I	M-, C	heme, Cu: Redox
II	26	II	M, C	Cu: Redox, H^+(?)
III	30	III	M, C	H^+(?)
	Large Nuclear Subunits			
V	17	IV	M, C	Assembly
VI	12	Va	M, C	
IV	11	Vb	M, C	Zn(?) 3SlN
	9	VIa	M, C	heart, muscle
No equivalent				= liver, kidney
	10	VIb	?, C-	
to VI in yeast				
	8	VIc	?, C	
	Small Nuclear Subunits			
VII(?)	6	VIIa	?, C	
VIIa(?)	6	VIIb	?, C	Assembly/stability
VIII	6	VIIc	?, C-	null mutants active
	5	VIII	?, C	

[1] Notation of Kadenbach (ref. 1)
[2] M = inner surface; C = outer surface; - = not exposed; ? not established

molecular mass is 204 kDa and about one-half of this is embedded in the mitochondrial membrane with most of the remainder projecting away from the outer surface (Fig. 1). The mass of the enzyme is partitioned amongst a number of subunits. Until very recently the subunit composition of the enzyme had been controversial but there now appears to be general agreement that the bovine enzyme is composed of thirteen subunits ranging in mass from 50,000 daltons to 6,000 daltons (Table 1; ref. 1). The three largest subunits (I-III) are the gene products of mitochondrial DNA and carry the redox active metal centers and, apparently, the proton translocation apparatus. The remaining subunits (IV-VIII) are substantially smaller, are coded for by the nuclear DNA, and, at least in some instances, appear to be involved in the assembly and stability of the mature enzyme complex. Most of these subunits appear to completely (M, C; Table I) traverse the membrane although some (M-, C-) are accessible from only one side.

It now appears that, in addition to the well-known and redox-active metal ions iron and copper, cytochrome oxidase contains 1 atom of zinc (ref. 2). The environment of this zinc constituent has recently been examined by exafs (ref. 3) which suggests that this ion is in a distorted tetrahedral environment provided by 3S (at 2.4 Å) and 1 N(O) at 2.08 Å. From a consideration of amino-acid sequences and secondary structure predictions subunit Vb (Table I) was proposed as the site of zinc binding (ref. 3). Partial depletion of the zinc does not affect catalytic activity.

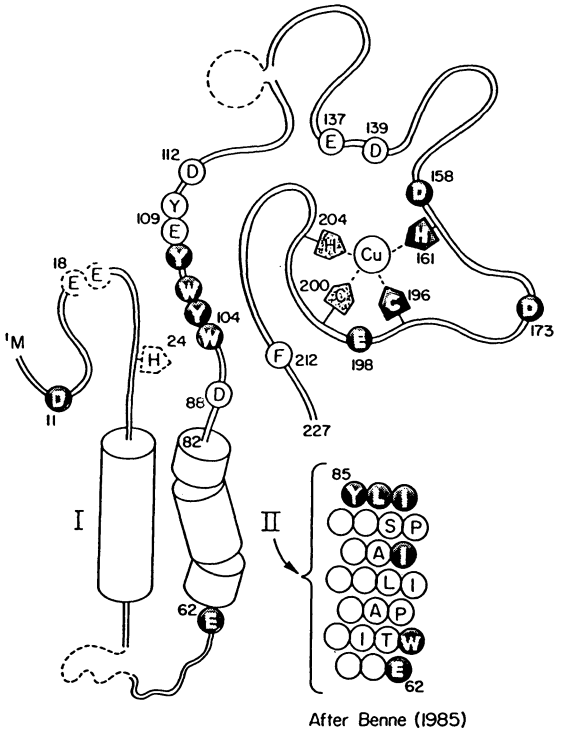
PROPERTIES OF THE PROSTHETIC GROUPS

General

The presence of heme a, rather than the more familiar heme b, as the iron-containing prosthetic group has several important consequences most of which are a result of a change in the spectroscopic (effective) symmetry. The replacement of the vinyl on pyrrole I by a farnesyl substituent and the methyl on pyrrole IV by a formyl group leads to a reduction in symmetry from four-fold (as found in protoheme derivatives) to less than three-fold. As a consequence the visible spectrum no longer shows the classic "alpha-beta" pattern found, for example, in reduced cytochrome c but is much more "chlorophyll-like" with a weak beta band and an intensified alpha-band which is red-shifted to ca 605 nm (ref. 4). Furthermore, the classic MCD A-term found in protoheme derivatives is absent in the corresponding heme a compounds; the presence of this transition requires that the symmetry be at least three-fold (thus ensuring the degeneracy of the lowest unoccupied porphyrin π orbitals of E symmetry). Likewise the intensity of the C-term present in the Soret MCD of ferric low-spin compounds is much weakened in the heme a series, again a result of the decrease in symmetry (ref. 5). This reduction in symmetry is

also apparent in the resonance Raman spectra and leads to a decrease in the magnitude of the polarization ratio of certain Raman lines (ref. 6). For example, Band IV (the core-size marker) has a polarization ratio approaching infinity in protoheme compounds while it is only unity in heme a compounds.

The four redox active metals can be divided into two pairs. Cytochrome a and Cu_A appear to be structurally distinct entities but function together in the sense that they accumulate electrons from cytochrome c and then act as a two-electron donor to the second pair of metal ions, namely cytochrome a_3 and Cu_B. This second pair is believed to be present at a binuclear center which constitutes the catalytic site where oxygen is reduced to water.



After Benne (1985)

Fig. 2

It is generally agreed that all four metal ions are confined to subunits I and II. The location of Cu_A in subunit II was originally based upon the similarity of part of the amino acid sequence of this subunit with the copper binding site of Type I copper proteins. This sequence, residues 161-204 (Fig. 2, ref. 7), contains the only two cysteine groups in the polypeptide, together with two histidines, in a short, highly-conserved stretch of polypeptide. Until recently it was believed that histidine 24 was also highly conserved. As both heme centers are coordinated by histidine it was thus possible that one of the two hemes was partially (a) or wholly (a_3) attached to this subunit. However, the sequence of the gene for this subunit from trypanosomes lacks histidine at this location (ref. 7). It therefore appears that both heme centers are located in subunit I. If, as has been suggested, there is also a sulfur atom in the coordination of Cu_B, then this metal ion is also present in subunit I for it is most probable that both cysteines of subunit II are coordinated to Cu_A.

Cytochrome *a*

Cytochrome a is the simplest of the four redox-active components. Epr, optical and mcd spectra all support the picture that this is a low-spin bis-imidazole derivative. The epr parameters fall nicely into the cytochrome b_5 domain of the Blumberg-Peisach "Truth Diagram" (ref. 8), while the visible and near-ir mcd spectra are strikingly similar to those of bis-imidazole heme a model compounds (ref. 9,10). The presence of at least one histidine as heme ligand has been established by endor measurements on [1, 3-^{15}N] histidine substituted enzyme (ref. 11).

Several useful features in the Raman spectrum have been assigned to this center (i) the line at 265 cm^{-1} has been attributed to a Fe-pyrolle tilt (ref. 12) (ii) the feature at 1588 cm^{-1} to the porphyrin coresize marker (ref. 6) and (iii) the feature at 1650 cm^{-1} to the formyl group (ref. 13).

A second low-spin species is formed reversibly upon raising and lowering the pH; it has the epr characteristics of a hydroxide derivative. It was originally thought that this high pH species was produced by a structural change at cytochrome $\underline{a}_3$ such that the original distal ligand had been replaced by hydroxide. However, the epr intensity of this component is clearly inversely correlated with the epr intensity of cytochrome $\underline{a}$ as the pH is changed, and this correlation is unaffected by the presence of cyanide which irreversibly traps cytochrome $\underline{a}_3$. It thus seems likely that this hydroxide species is a derivative of cytochrome $\underline{a}$ (ref. 14).

Copper A

Although Cu_A was the first component of cytochrome oxidase to be visualized by epr the interpretation of its unusual epr spectrum, and the understanding of the underlying structure, has yet to be satisfactorily completed. The facts are simple: The epr has a simple axial lineshape at X-band ($g_x = g_y = 2.00$, $g_z = 2.18$) though spectra at Q-band (35 GH_z) show a rhombic splitting ($g_x = 1.99$, $g_y = 2.02$) (ref. 15); no copper hyperfine is apparent at either 9 or 35 GH_z and the intensity of the epr signal only accounts for ca 0.8 Cu atoms per $\underline{a} + \underline{a}_3$.

There are several estimates of the magnitude of the copper hyperfine interaction. Epr spectra taken at very low frequencies (which reduces the contributions to the linewidth due to g-strain) exhibit a number of shoulders and inflections which were taken to represent contributions from the copper hyperfine interactions. An attempt to rationalize these features led to estimates of 10 (x), 45 (y) and < 40 (z) gauss for the components of A (ref. 16). Independent endor measurements suggest values of 24, 38 and 32 gauss for the hyperfine components (ref. 17); preliminary analysis of pulse field-sweep epr data is consistent with the endor values (ref. 18) but this analysis needs to be refined. While these sets of values are only in qualitative agreement they make the points that the values for A are extremely small and, at least in the case of the endor experiment, much closer to isotropic than is normally the case. (The corresponding hyperfine values for plastocyanin are < 17, < 17, and 63 gauss). In plastocyanin the geometry is a flattened tetrahedron provided by two histidines plus a cysteine which lie slightly below the xy plane with a single distant methionine at the apex. Solomon (ref. 19) has calculated that, for plastocyanin, the magnitude and anisotropy of the copper hyperfine values signify that the electron is only about 40% localized on the metal ion. However, the intrinsic mcd of Cu_A is both qualitatively and quantitatively different from that of a Type I copper center (ref. 20) and, importantly, does not show any evidence for the traditional 600 nm band of "blue copper". As mcd reflects the intrinsic property of the parent chromophore this result is additional evidence of a fundamental difference between these two species of copper.

Oxidase obtained from yeast auxotrophs specific for either cysteine or histidine has been substituted with either deuterium in the beta methylene of cysteine or ^{15}N-histidine (ref. 21). Both isotopically substituted derivatives of the enzyme showed clear changes in the endor of Cu_A providing very good evidence that these two amino acids are in the coordination sphere of this metal ion. The issue of whether there are one or two sulfur-containing amino acids in the coordination is not resolved. The amino acid sequence in the crucial region contains two invariant cysteine residues (Fig. 2) and a recent comparison of the exafs pattern of native enzyme with that obtained from enzyme that has been specifically depleted of Cu_A has led to the conclusion that two sulfur atoms are indeed coordinated to this metal ion (ref. 22).

The linear electric field effect (the shift of the electron g-values in response to an applied electric field) exhibited by Cu_A is quite unlike that found with any other copper compound while the decay of the electron spin-echo does not show the typically deep modulation pattern exhibited by copper-histidine compounds. It was consequently concluded (ref. 23) that histidine could not be ligand to the copper ion--which it undoubtedly is--unless the unpaired electron was not on the copper ion.

Taken together these facts support a picture of a very highly covalent copper center in which a large fraction of the unpaired electron is delocalized onto one or, more likely, two sulfur ligands. One can make an estimate of the degree of delocalization of this unpaired electron using the standard expressions for the hyperfine interaction (ref. 24). If we assume that this interaction is isotropic--an approximation to the endor measurements--a^2, the fraction of electron on the metal ion, is about 0.2.[1] Such an ascription suggests that the RS-Cu(I) proposal made some years ago by Peisach and Blumberg (ref. 25) is, in fact, not so far from the truth, though a molecular orbital picture in which the paramagnetic electron is distributed over the metal ion and the several ligands is almost certain to be the ultimate description. This high degree of delocalization is consistent with the low energy and low intensity of the 800 nm optical band widely regarded to be due to Cu_A.

The low recovery of epr intensity for this species is not explained. Two obvious possibilities are (i) the signal arises from a system which has a thermally accessible epr-invisible excited state. If this is the case then this excited state must be within a few degrees of the ground state for low quantitations are still obtained at liquid helium temperatures. (ii) The resonance is partially broadened by long-range dipolar coupling to a second paramagnet (ref. 26). This second paramagnet cannot be cytochrome $\underline{a}_3$ or Cu_B for the epr of Cu_A is unchanged in a partially reduced, carbon monoxide derivative in which these two centers are diamagnetic. Although this second possibility is superficially plausible, the independence of lineshape and intensity on temperature places constraints on the spin-lattice relaxation time of the second paramagnet (ref. 26) which would seem to be difficult, if not impossible, to meet in practice. This explanation for the low epr intensity must therefore be viewed with suspicion.

Cytochrome a_3 and Cu_B: the binuclear center

The first direct evidence that cytochrome $\underline{a}$ and Cu_B were present in a binuclear complex was provided by magnetic susceptibility measurements on the isolated enzyme (ref. 27). These data showed that the combined magnetism of these two metal ions was appropriate to a S = 2 center and this was interpreted in terms of an antiferromagnetic interaction between high-spin ferric $\underline{a}_3$ and the S = $\frac{1}{2}$ copper ion.

The immediate environment about each metal ion is not well characterized. Using NO as a probe of heme proximal ligation it has been established that histidine is coordinated to the heme center (ref. 28). Furthermore, the location of Band IV in the Raman spectrum indicates that $\underline{a}_3$ is six-coordinate (ref. 6,29); however, the identity of the sixth ligand is not known. Even less is known about Cu_B. Originally, exafs data suggested that the coordination be 4(N,O) (ref. 30), a conclusion consistent with endor measurements (ref. 31) which revealed the presence of 3 nitrogens ligated to the metal. However, epr spectra of Cu_B present in alkaline-treated enzyme were interpreted using 2N2O ligation (ref. 14). More recent exafs experiments on enzyme depleted of Cu_A has been interpreted by Scott (ref. 22) as indicating a ligation pattern of 3(N,O)1S, a conclusion supported by results of similar experiments reported by Powers *et al.* (ref. 32). However, in both cases, the procedures used for copper depletion were brutal and more data is needed before we can assert unequivocally that the depletion procedures have not led to some overall structural changes in the protein with rearrangement in the coordination of this copper ion.

Because of the presence of the antiferromagnetic interaction between these two metal centers the presence of a bridging ligand has long been suspected. However, the identity of such a ligand has not been established. There were early suggestions that S was situated between the iron and copper but this proposal (ref. 33) seems not to have survived the passage of time. Furthermore, the distance between the two metal centers is not established.

---

[1] The calculation is only approximate for it makes no allowance for the contribution of the sulfur-centered electron to the orbital magnetism. However, even when one uses traditional copper g-values the value of a^2 does not exceed 0.3. In either case one finds that the electron is primarily confined to the ligand atoms(s).

One exafs group insists that this distance is 3.75 Å (ref. 33) while a second laboratory maintains, with equal conviction, that the distance is much smaller, specifically 3.0 Å (ref. 31); this latter group does not find any features in their Fourier-transformed exafs data which suggests the presence of a heavy atom at 3.75 Å. At the same time the Van der Waals distance between Fe and Cu independently ligated by N (or O) is greater than 4 Å, larger than any distance deduced from exafs measurements, and hardly likely to propagate the strength of the antiferromagnetic exchange interaction that is observed.

Much of the difficulty in studying these two centers is the absence of convenient techniques. Cu_B has been observed by epr during catalytic turnover (ref. 34,35) and in enzyme maintained at high pH (ref. 14) but no optical or Raman characteristics of this center have been identified. Cytochrome $\underline{a}_3$ can be studied by optical and mcd methods using its near infra-red transition at 655 nm but this band is weak and poorly resolved. It can also be studied by Raman spectroscopy having characteristic features between 1300-1700 cm^{-1}, where many of the vibrational modes of porphyrin are found, and at ca 220 cm^{-1} where the $\underline{a}_3$ iron-proximal histidine stretch is located (ref. 35). Cytochrome $\underline{a}_3$ can also be detected via its g = 6 epr signal in enzyme in which Cu_B has been reduced e.g., during redox titrations (ref. 37).

SPATIAL RELATIONSHIPS OF THE FOUR REDOX CENTERS

The geometric relationships between the redox centers has been arrived at indirectly from the analysis of epr relaxation and static broadening data and a synthesis of results obtained from several laboratories can be summarized thus:

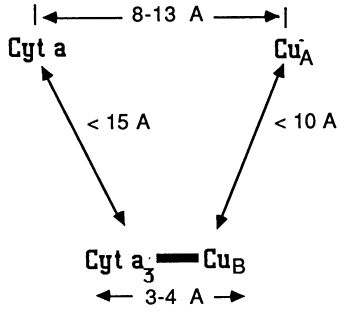


Fig. 3

UNUSUAL EPR SIGNALS PRESENT IN CYTOCHROME OXIDASE

A variety of unusual epr signals can be observed with this enzyme. Some of these signals are reproducible while others are not. Perhaps the best known of these is the g = 6 signal which appears on partial reduction of the enzyme and which is generally acknowledged to arise from that fraction of partially reduced molecules in which the binuclear center has the composition $Cu_B(I)$-Fe(III); the diamagnetism of the copper results in the loss of the antiferromagnetic interaction and the appearance of the typical epr of high-spin heme, viz cytochrome $\underline{a}_3$ (ref. 37).

A more enigmatic signal is the g' = 12 resonance, a species long acknowledged to be an erratic component of the enzyme. It is now established that this signal is due to a conformational form of the enzyme in which the binuclear center reacts sluggishly with cyanide (the so-called 'slow' form of oxidase) (ref. 38); this form of the enzyme appears to be a preparation artefact as it is not observed in the membrane and is observed to appear in some preparations of the enzyme as the purification proceeds (ref. 38). When present, the signal is abolished by cyanide under conditions where cytochrome $\underline{a}$ and Cu_A are unaffected. It thus seems clear that the signal is a property of the binuclear center. Furthermore, the signal is intensified under the special epr configuration of having the microwave radiation parallel to the applied magnetic field (ref. 39) implying that the transition is not a conventional $\Delta m_s = \pm 1$ and represents epr between two levels with even spin. The components of the S = 2 binuclear center is the obvious choice and two possibilities arise (i) the g' = 12 signal arises from transitions between

the $\Delta m_S = \pm 1$ components of the S = 2 state (ref. 39) or (ii) the signal arises from transitions between the ±2 sub-levels (ref. 38). In both cases the transitions are forbidden in rigorously axial symmetry and are only "turned-on" by rhombic distortions; first-order rhombic distortions are sufficient for the ±1 states, second-order terms are needed for the ±2 states (ref. 39). Both transitions can occur in the g' = 12 region as can the corresponding transitions of a high-spin S = 2 Fe(IV) ion (ref. 40). Simulations show that the transitions within the ±1 levels of a coupled S = 2 system best reproduces the observed epr at two frequencies. [It should be noted that the magnetic behavior of S = 2 states arising from (i) mononuclear or (ii) exchange-coupled binuclear centers are quite different with terms in the exchange coupling tensor appearing only in the latter (ref. 39)] .

Another unusual epr signal is the g' = 5 species which occurs together with associated features at g = 1.78 and 1.69. This species is formed within milliseconds following mixing fully-reduced oxidase with dioxygen, at a time consistent with complete reoxidation of the enzyme (ref. 41). The signal is relatively stable and can persist for many minutes. Indeed, it has been observed as a minority component in at least one enzyme preparation as isolated (ref. 42). The signal is quite temperature dependent and disappears below 10 K (ref. 43) providing strong evidence that it arises from an excited state. This fact, taken with its "non-classical" shape, suggests that, like the g' = 12 signal, the g' = 5 signal arises from an excited state of the S = 2 binuclear center. A similar comment might apply to the broad signal induced upon addition of fluoride to the resting enzyme (ref. 44).

Finally, there is the very unusual copper signal which is observed together with Cu_A when the reduced enzyme is reoxidized with dioxygen under very carefully controlled conditions (ref. 35). This signal exhibits well resolved copper hyperfine splitting (138 gauss) at $g_{\parallel} = 2.25$; $g_{\perp}$ is not apparent in the spectrum. It was suggested (ref. 35) that the signal arises from an exchange interaction between Cu_B(II) and the $\Delta m_S = \pm 1$ levels of Fe(IV). Such an interaction could shift g_x and g_y to very high fields while leaving g_z essentially unaffected (ref. 45).

MECHANISM OF REACTION

The details of the overall mechanism of action are now becoming much clearer. In the simplest scenario the enzyme is converted to the fully reduced form by successive reaction with four equivalents of ferrocytochrome c. Curiously, even this is not a straightforward process. Although the reduction of Cu_A and cytochrome a is extremely rapid, subsequent electron transfer to cytochrome a_3 is orders of magnitude slower than can support the overall catalytic reaction. A plausible explanation (ref. 46) for this surprising result appears to reside in the dual circumstances that the electron transfer to the binuclear center is a coordinated two-electron process while, because of the redox linkage between the two cytochromes, the electron affinity of the oxidized binuclear center is low. As a consequence the net rate of reduction of a_3 is controlled by the small, equilibrium, fraction of ferricytochrome a in two-electron reduced enzyme, this quantity determining the rate of addition of the third and fourth electrons.

The rate of reaction of dioxygen with fully reduced enzyme is very fast and appears to be complete within 20 usec under normal laboratory conditions. Time-resolved resonance Raman studies (ref. 47) suggest that the immediate product of this reaction is the dioxygen adduct of cytochrome a_3(II). Over the next 100 usec this species is converted into a state in which a_3 is oxidized; in all likelihood to a μ-peroxy Fe(III)-Cu(II) species. The remaining two electrons are transferred (from cytochrome a and Cu_A) during the next few milliseconds. There is some evidence that either metal center can transfer its electron directly to a_3-Cu_B although, in view of the rapid electron equilibration between these two centers, I do not find the data particularly convincing. It does appear however that, at cryogenic temperatures, the transfer of these two electrons can be separated in time so that it is possible to obtain finite amounts of species in which 3 electrons have been transferred to oxygen. The species first formed has been postulated to be a Fe(II)-Cu_B(II)-hydroperoxide; this subsequently reacts with cleavage of the O-O bond, elimination of oxide and forming a complex containing Fe(IV)=O and Cu_B(II). The hydroperoxide being six-coordinate, presumably contains the diamagnetic low-spin Fe(II) and thus should exhibit an essentially normal Cu(II) signal, perhaps the rhombic spectrum reported by

Reinhammer et al. (ref. 34). (It is likely that a hydroxide-bridged Fe(II)-Cu(II) species would also have a normal copper epr spectrum, for the same reason.) The Fe(IV) species could well be responsible for the unusual Cu signal referred to in the last section. The transfer of the final electron leads to the expulsion of the second oxide and restoration of the four metal centers to their original oxidation--and possibly spin--states.

At issue, however, is whether the oxidized species formed upon reaction with dioxygen (often called the 'pulsed' enzyme) is identical with the enzyme before the cycle of reduction and oxidation. Much of the early data which suggested that these species were different is now known to be false, a circumstance which can be traced to the formation of hydrogen peroxide adducts of oxidized enzyme. However, many of the enzyme preparations in common use contain large proportions of the g' = 12 (the 'slow') form of oxidase. With these preparations the reoxidation product is clearly different from the starting material and appears to be the g'12-less (i.e., the "rapidly reacting") form; this subsequently reverts to the g'12 species. By contrast the reoxidized enzyme obtained starting with the g'12-less form of the enzyme is very similar to the original species. As the g'12 containing species seems to be a product of the laboratory (ref. 38) any conclusions drawn from its use must be examined with circumspection.

PROTON PUMPING FUNCTION OF CYTOCHROME OXIDASE

As the details of the structure of the redox centers and the mechanism of oxygen reduction are being established, attention is turning to the second catalytic property of cytochrome oxidase, its ability to couple electron transfer to the creation (and maintenance) of a proton electrochemical gradient (ref. 48).

There are two schools of thought in this area. The first assumes that the site of translocation is remote from the pertinent redox center, and pictures the act of electron transfer as inducing some structural strain in the enzyme complex. This strain serves to transmit the redox free energy to the proton translocation site. In this "conformational change" model for energy conservation attention is paid to identifying the protein subunit which serves as the proton translocating device. This school was particularly encouraged by early data which suggested that subunit III was the proton translocase with (i) modification of this subunit by dicyclohexylcarbodiimide, a reagent which is a specific inhibitor of proton translocation in the ATP synthetase, leading to loss of translocation and, to a lesser extent, electron transfer; and (ii) enzyme depleted of subunit III appeared to be competent in electron transfer but not proton translocation. However this last result is not reproducible, some groups finding significant proton translocation in oxidase which has been depleted of subunit III (ref. 49) and it now appears that this subunit does not function directly as a proton translocator but possibly serves to control the proton permeability of the membrane (ref. 50) such that, when it is absent, proton gradients formed by electron transfer collapse too rapidly to be observed by conventional methodology. In the conformational coupling scenario the mechanism of coupling between the electron and proton movements is not easy to define and is deliberately left quite vague.

The second school of thought believes that proton translocation is intimately involved with the act of electron transfer, most plausibly via a ligand-conduction mechanism. In a typical scheme the sequence of reduction and reoxidation of a specific metal center is accompanied by the protonation and deprotonation of a ligand to that metal center (or, alternatively to an adjacent amino acid residue) with the proton being captured at the inner surface of the membrane and released at the outer surface. Members of this school have various prejudices as to the specific metal ion which implements the proton translocation with both cytochrome a and Cu_A being strong favorites. A recent variant of this class invokes ligand exchange at Cu_A as the fundamental device (ref. 51). In this proposal, reduction of Cu_A is accompanied by ligand exchange with cysteine being replaced by a second, highly basic, amino acid. These two amino acids are proposed to be on opposite sides of the membrane. Cysteine release leads to proton capture by the mercaptide from the inner surface of the membrane and coordination of the second amino acid leading to proton release at the outer surface. Reoxidation of the metal ion leads to restoration of the original coordination configuration but with the essential constraint that the proton released by the cysteine is not returned to the medium but is captured by the basic amino acid that is displaced.

Upon examination of the peptide sequences of subunit II (ref. 7) one finds that tyrosine is the only basic amino acid conserved across all species. There are three tyrosine residues but one of them is confined to transmembrane helix II (Fig. 2) and is thus unlikely to be available. The other two are clustered close to residue 105, and are thus far removed from the metal binding site, at least as far as primary sequence is concerned. Moreover, hydropathy analysis suggests that the - COOH terminal segment running from residues 88 to 227 (Fig. 2) is on one side of the membrane. Because subunit III has been identified as the binding site for cytochrome c this side must be the exterior surface. Thus the proposed mechanism would require a proton channel which traverses the whole membrane for which there is no evidence. Furthermore, it is hard to understand why cysteine should be displaced from the cuprous species as postulated (ref. 51), the conventional wisdom being that the reduced metal ion should have greater affinity for sulfur ligation.

Schemes such as that just described are relatively easy to devise; however, making them unidirectional invariably requires some ad hoc assertions about the properties of the proton-translocating center. Nevertheless, it is very gratifying that we have progressed to the stage where concrete mechanisms of this kind can be proposed and, hopefully, even tested in the not too-distant future. Thus, even though, as this review documents, there are serious gaps in our detailed picture of the architecture of cytochrome oxidase, it is clear that there is now a sufficient structural base upon which meaningful experiments can be predicated. Such schemes will undoubtedly be revised and refined as the structural information on the individual redox centers is completed and we can now look forward to a period in which the elements of the mechanism of proton translocation and its relationship to electron transport will be established.

Acknowledgments

I wish to acknowledge the efforts of my collaborators and the many conversations with colleagues in the field. Preparation of this review was supported by the National Institutes of Health (GM 21337) and the Welch Foundation (C-636).

REFERENCES

1. B. Kadenbach, J. Jaurasch, R. Hartmann and P. Merle, Anal. Biochem., 129, 517 (1983).
2. O. Einarsdottir and W.S. Caughey, Biochem. Biophys. Res. Comm., 124, 836 (1984).
3. A. Naqui, L. Powers, M. Lundeen and B. Chance, Biophys. J., 51, 312a (1987).
4. M. Gouterman, J. Mol. Spectrosc., 6, 138 (1961).
5. M.A. Livshitz, A.M. Arutyan and Y.A. Sharanov, J. Chem. Phys., 64, 1276 (1976).
6. W.H. Woodruff, R.J. Kessler, N.J. Farris, R.F. Dallinger, K.R. Carter, T.A. Antalis and G. Palmer, in "Electrochemical and Spectro-Electrochemical Characteristics of Biological Redox Compounds" (Kadish, K. ed), Advances in Chemistry, 201, 626 (1982).
7. R. Benne, Trends in Genetics, 1, 117 (1985).
8. G.T. Babcock, J. van Steelandt, G. Palmer, L.E. Vickery and I. Salmeen, in "Cytochrome Oxidase" (T.E. King, Y. Orii, B. Chance and K. Okunuki, eds), Developments in Biochemistry, 5, 105 (1979).
9. K. Carter and G. Palmer, J. Biol. Chem., 257, 13507 (1982).
10. D.G. Eglington, M.K. Johnson, A.J. Thompson, P.E. Gooding and C. Greenwood, Biochem. J., 191, 319 (1980).
11. C.T. Martin, C.P. Scholes and S.I. Chan, J. Biol. Chem., 260, 2857 (1985).
12. S. Choi, J.J. Lee, Y.H. Wei and T. Spiro, J. Am. Chem. Soc., 105, 3692 (1983).
13. I. Salmeen, L. Rimai and G.T. Babcock, Biochemistry, 17, 800 (1978).
14. G.T. Baker and G. Palmer, Biochemistry, in press (1987).
15. R. Aasa, S.P.J. Albracht, K.E. Falk, B. Lanne and T. Vanngard, Biochim. Biophys. Acta, 422, 260 (1976).
16. W. Froncisz, C.P. Scholes, J.S. Hyde, Y.-H. Wei, T.E. King, R.W. Shaw and H. Beinert, J. Biol. Chem., 254, 7482 (1979).
17. B. Hoffman, J.E. Roberts, M. Swanson, S.H. Speck and E. Margoliash, Proc. Natl. Acad. Sci. U.S.A., 77, 1452 (1980).
18. C.P. Scholes, C. Fan and H. Taylor, Biophys. J., 51, 76a (1987).
19. K.W. Penfield, A.A. Gewirth and E.I. Solomon, J. Am. Chem. Soc., 107, 4519 (1985).
20. A.J. Thompson, C. Greenwood, J. Peterson and C.P. Barret, J. Inorg. Chem., 28, 195 (1986).

21. T.H. Stevens, G.T. Martin, H. Wang, G.W. Brudwig, C.P. Scholes and S.I. Chan, J. Biol. Chem., 257, 12106 (1982).
22. P.M. Li, J. Gelles, S.I. Chan, R.J. Sullivan and R.A. Scott, Biochemistry, in press (1987).
23. W.B. Mims, J. Peisach, R.W. Shaw and H. Beinert, J. Biol. Chem., 255, 6843 (1980).
24. B.R. McGarvey, J. Phys. Chem., 71, 51 (1967).
25. J. Peisach and W.E. Blumberg, Arch. Biochem. Biophys., 165, 691 (1974).
26. J.S. Leigh, J. Chem. Phys., 52, 2608 (1970).
27. M. Tweedle, L.J. Wilson, L. Garcia-Iniguez, G.T. Babcock and G. Palmer, J. Biol. Chem., 253, 8065 (1978).
28. T.H. Stevens and S.I. Chan, J. Biol. Chem., 256, 1069 (1981).
29. G.T. Babcock, P.M. Callahan, M.R. Ondrias and I. Salmeen, Biochemistry, 20, 959 (1981).
30. R.A. Scott, J.R. Schwartz and S.P. Cramer, Biochemistry, 25, 5546 (1986).
31. J. Cline, B. Reinhammer, P. Jensen, R. Venters and B. Hoffman, J. Biol. Chem., 258, 5124 (1983).
32. L.F. Powers, Y. Ching, B. Chance and D.C. Wharton, Biophys. J., 51, 300a (1987).
33. L. Powers, B. Chance, Y. Ching and P. Angiolillo, Biophys. J., 34, 465 (1981).
34. B. Reinhammer, R. Malkin, P. Jensen, B. Karlsson, L.-E. Andreasson, R. Aasa, T. Vanngard and B.G. Malmstrom, J. Biol. Chem., 255, 5000 (1980).
35. B. Karlsson, R. Aasa, T. Vanngard and B.G. Malmstrom, FEBS Lett., 131, 186 (1981).
36. J. Schoonover, R. Dye, W.H. Woodruff, G.T. Baker and G. Palmer, Biochemistry, to be submitted.
37. B.F. van Gelder and H. Beinert, Biochim. Biophys. Acta, 189, 1 (1969).
38. G.T. Baker, M. Noguchi and G. Palmer, J. Biol. Chem., 262, 595 (1987).
39. W.R. Hagen, W.R. Dunham, R.H. Sands, R.W. Shaw and H. Beinert, Biochim. Biophys. Acta, 765, 399 (1984).
40. G.W. Brudwig, R.H. Morse and S.I. Chan, J. Mag. Res., 67, 189 (1986).
41. R.W. Shaw, R.E. Hansen and H. Beinert, Biochim. Biophys. Acta, 548, 386 (1979).
42. L. Young and G. Palmer (unpublished data).
43. W.R. Dunham, R.H. Sands, R.W. Shaw and H. Beinert, Biochim. Biophys. Acta, 784, 73 (1983).
44. G.T. Brudwig, T.H. Stevens, R.H. Morse and S.I. Chan, Biochemistry, 20, 3912 (1981).
45. J.A. Christner, E. Munck, T.A. Kent, P.A. Janick, J.C. Salerno and L.M. Siegel, J. Am. Chem. Soc., 106, 6786 (1984).
46. D. Bickar, J.F. Turrens and A.L. Lehninger, J. Biol. Chem., 261, 14461 (1986).
47. G.T. Babcock, J.M. Jean, L.N. Johnston, W.H. Woodruff and G. Palmer, J. Am. Chem. Soc., 106, 8305 (1984).
48. B.G. Malmstrom, Biochim. Biophys. Acta, 811, 1 (1985).
49. M. Finel and M. Wikstrom, Biochim. Biophys. Acta, 851, 99 (1986).
50. P. Sarti, M.G. Jones, G. Antonini, F. Malatesta, A. Colosimo, M.T. Wilson and M. Brunori, Proc. Natl. Acad. Sci. U.S.A., 82, 4876 (19).
51. J. Gelles, D.F. Blair and S.I. Chan, Biochim. Biophys. Acta, in press.