Hypothesis

STRUCTURAL MODELS FOR THE HEME a_3/COPPER ACTIVE SITE OF CYTOCHROME *c* OXIDASE

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1. Introduction

Cytochrome *c* oxidase is a complex, seven subunit enzyme containing two copper atoms, Cu_A and Cu_B, and two hemes, the low-spin heme *a* and the high-spin heme a_3 (fig.1). Located at the terminus of the mitochondrial respiratory chain it has the crucial role of catalysing the rapid, four electron reduction of molecular oxygen to water (reviewed [1a,b]). The oxygen binding site is known from abundant spectroscopic and chemical evidence to be at the heme a_3. An intriguing structural aspect of this active site in its oxidized form is the strong antiferromagnetic coupling between the presumed high-spin $S = 5/2$ Fe(III) heme a_3 and the $S = 1/2$ Cu_B(II) atom making these two metals electron paramagnetic resonance (EPR) 'invisible' and lowering their magnetic susceptibilities [2,3]. The strength of this antiferromagnetic coupling is quantified by $-J$, the exchange coupling constant, which is >200 cm^{-1} for the oxidase [4]. The heme a_3 and Cu_B are assumed to be in close proximity and bridged by a ligand which can mediate very strong magnetic exchange. Since the structure of the active site must be known in order to understand the catalytic mechanism there have been numerous suggestions regarding its nature. This communication briefly, but critically, analyses existing proposals in the light of recent model compound studies and concludes by discussing a model prejudiced in favor of oxygen bridged species.

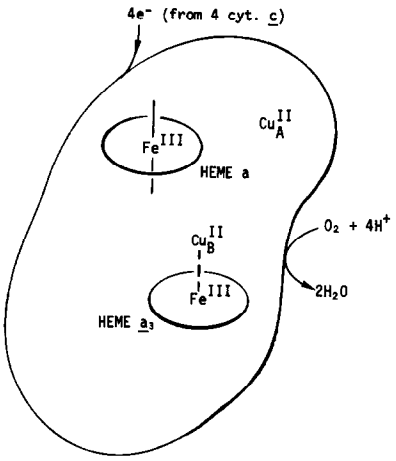

Fig.1. Schematic representation of cytochrome *c* oxidase in its oxidized form. The hemes are represented as ellipses. Heme *a* is low-spin and heme a_3 is high-spin.

2. Discussion

2.1. *The imidazolate model*

The proposal of a Cu_B(II)–imidazolate–Fe(III) heme a_3 structure using a deprotonated axial histidine [5] (fig.2a) has been strongly adopted by Palmer [3]. Supportive evidence comes from several hemoglobin-like properties of heme a_3 particularly axial N EPR hyperfine splitting in the NO adduct and also circumstantially from the probable existence of a Cu–imidazolate–Zn bridge in a superoxide dismutase. We have recently synthesized a variety of imidazolate bridged metalloporphyrins [6] all of which show that imidazolate is a rather poor mediator of antiferromagnetic coupling. One further new result, which at first sight seems far removed from an oxidase model,

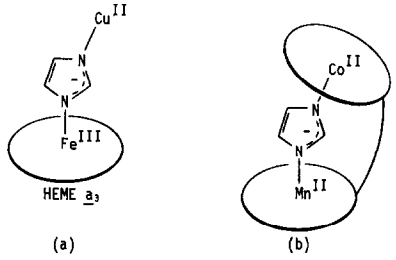

Fig.2. (a) The imidazolate model [3]. (b) A Co(II)–imidazolate–MN(II) face-to-face porphyrin species using the porphyrin reported in [6].

is that the Mn(II)–imidazolate–Co(II) species in fig.2b has $-J = 5$ cm^{-1} [7]. Despite the different metals this is the first test of imidazolate coupling between an $S = 5/2$ metal (like Fe(III) and an $S = 1/2$ metal (like Cu(II)) and moreover, the orbital occupations are very closely related to what we expect in an authentic Fe–Cu model [8]. Such low values of $-J$, taken together with those of other workers [9–11] for Cu(II) imidazolate dimers ($-J = 25$–90 cm^{-1}) and all definitively characterized species [6,12] suggest that imidazolate is not capable of mediating the strong coupling observed in cytochrome oxidase (>200). Cyanide-treated oxidase [4] has $-J = 60$ cm^{-1} which does fall into the range of known values in model systems. However, consideration of the probable mismatch of mutual orbital symmetries of the unpaired electrons (π on Fe and σ on Cu) leads us to predict that this low-spin Fe(III)–imidazolate–Cu(II) situation would have very small or negligible antiferromagnetic coupling.

2.2. *Various sulfur models*

Recent X-ray evidence pointing towards a Cu(I) oxidation level in the oxidized enzyme [13] has revived discussion on the possibilities of cysteinate radical [14], a Cu(III) state, and other sulfur ligands [15]. If only because there is a total lack of precedent for such structures in metalloproteins we tend to disfavor them.

2.3. *The bridging carboxylate model*

Seiter [16] has suggested that a carboxylate group might bridge Cu_B and heme a_3. This is an attractive proposal because there is good inorganic precedent for strong antiferromagnetic coupling at least in dicarboxylate dimers and also there is bridging ligand precedent in hemerythrin. Proton transfer from a carboxylic acid to oxygen during reduction offers an additional role to this group.

2.4. *The bridging oxygen model*

We now propose a bridging oxygen model (fig.3) which is consistent with all available experimental data. Some aspects of the model have their origin in previous hypotheses [17,18]. By assembling this model we can focus attention on what may turn out to be a rather simple picture of cytochrome oxidase and one which is based on sound inorganic chemical and biochemical principles. The resting, oxidized state of the enzyme, I (fig.3), has a μ-oxo Fe(III)–O–Cu(II) moiety. Strong antiferromagnetic coupling in Fe–O–Fe dimers ($-J = 95$–146 cm^{-1}) [19] and a diamagnetic Cu–O–Cu carbonate dimer [20] provide good precedent for the prediction that an Fe–O–Cu linkage, probably linear, could have $-J > 200$ cm^{-1}. Alteration of this bridge by various anions would lower (cyanide) or abolish (sulfide [21]) coupling. An axial histidine ligand is proposed in the sixth coordination site but quite possibly is not coordinated at

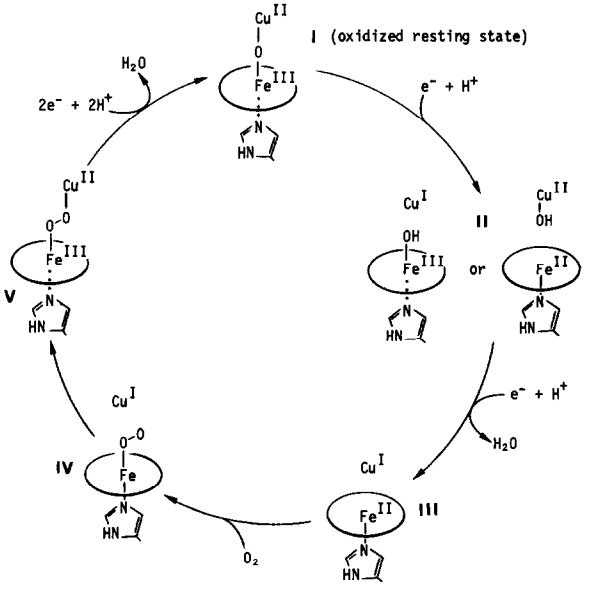

Fig.3. The bridging oxygen model. Heme a_3 is represented by the elipse.

this stage since ligation of μ-oxo ferric heme dimers is undocumented. Moreover, a mutant hemoglobin, Boston M, provides definitive biological precedent for proximal histidine dissociation accompanying 'distal' oxyanion coordination [22]. As reduction proceeds the oxide ligand will be expelled as a water molecule (fig.3). One electron reduction to state **II** destroys the antiferromagnetic coupling and provides an explanation for the observation of minor high-spin ferric heme signals and additional Cu intensity in the EPR spectra of partially reduced oxidase [16,23]. Further reduction to state **III** gives the necessary deoxyhemoglobin-like high-spin ferrous heme which can rapidly bind dioxygen to give state **IV**, a species identified at low temperature by Chance [18]. Since a vacant coordination site has probably been generated at Cu_B(I) it is reasonable to propose the μ-peroxo dimer **V**. There is good inorganic precedent for such species [24], hemocyanin and hemerythrin have this possibility, and also, a species proposed to be this has been observed at low temperature [18]. A further two electron reduction (the electrons being supplied by heme *a* and the other copper(I), Cu_A, both of which we consider to have a 'battery and wire' function) would extrude the second water molecule, balancing the overall reaction stoichiometry, and returning the enzyme to its resting oxidized state, **I**. Further attractive features of this mechanism are:

(i) The natural way the overall four electron reduction of dioxygen is broken down into at least three steps;

(ii) The obvious compatibility with very fast reaction kinetics;

(iii) The biological efficiency of having the reaction product as the bridging ligand.

In summary, we favor an oxygen-bridged species for the oxidized Cu_B(II)/Fe(III) heme a_3 active site of cytochrome *c* oxidase. While carboxylate oxygen or even hydroxide ion are viable candidates we presently prefer an oxide bridge as a working hypothesis in the design of experiments to elucidate the catalytic mechanism.

Acknowledgements

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