A wide range of spectroscopic and kinetic techniques have now been applied to probe the nature of the metal sites and the route of electron transfer. The consensus view is that electrons enter the complex through one of the haem *a* groups (cytochrome *a*) and are rapidly transferred to a copper atom (Cu_A). Electrons subsequently pass to a binuclear centre consisting of a copper atom (Cu_B) and a haem *a* group (cytochrome a_3) in close association and which act as the oxygen binding site. The properties of these metal sites and the nature of their immediate environments have now been partially elucidated [*e.g.* 8–10].

The mechanism of reaction and the nature of bound intermediates have been investigated by coupling spectroscopic methods with low temperature trapping techniques thus allowing intermediates with short life times at *in vivo* temperatures to be captured and studied [11, 12].

A short review of the structure of cytochrome *c* oxidase and the nature of the metal sites will be presented together with an outline of the catalytic cycle as postulated from recent EPR measurements [13]. Experiments were performed in which samples of the enzyme during 'turnover', and whilst being monitored by optical methods, were rapidly frozen and prepared for EPR spectroscopy. In this way the optical and EPR signals associated with the metal sites could be related to each other and to the level of reduction maintained during steady-state. This technique has yielded information regarding which of the many known derivatives of the enzyme are populated during catalysis.

Attention will also be drawn to site–site interactions and to recent proposals suggesting that interconversion between forms of the enzyme (possibly conformational variants) play a role in the regulation of activity [14].

1 M. Wikström, K. Krab and M. Saraste, 'Cytochrome Oxidase, A Synthesis', Academic Press, London, 1981.
2 M. Brunori, E. Antonini and M. T. Wilson, in 'Metal ions in Biological Systems', (H. Sigel, ed.) pp. 187–228, Marcel Dekker, New York, 1981.
3 B. G. Malmström, *Biochim. Biophys. Acta, 549,* 281 (1979).
4 N. W. Downer, N. C. Robinson and R. A. Capaldi, *Biochemistry, 15,* 2930 (1976).
5 G. J. Steffens and G. Buse in 'Cytochrome Oxidase', (T. E. King, Y. Orii, B. Chance and K. Okunuki, eds.) pp. 79–90, Elsevier/North Holland, Amsterdam (1979).
6 S. D. Fuller, R. A. Capaldi and R. Henderson, *J. Mol. Biol., 134,* 305 (1979).
7 T. G. Frey, M. J. Costello, B. Karlsson, J. C. Haselgrove and J. S. Leigh, *J. Mol. Biol., 162,* 113 (1982).
8 H. Beinert, R. Hansen and C. R. Hartzell, *Biochim. Biophys. Acta, 423,* 339 (1976).
9 T. H. Stevens, G. W. Brudwig, D. F. Bocian and S. I. Chan, *Proc. Natl. Acad. Sci. U.S.A., 76,* 3320 (1979).
10 L. Powers, B. Chance, Y. Ching and P. Angiolillo, *Biophys. J., 34,* 465 (1981).
11 B. Chance, C. Saronio and J. S. Leigh, *J. Biol. Chem., 250,* 9226 (1975).
12 G. M. Clore, L. E. Andreasson, B. Karlsson, R. Aasa and B. G. Malmström, *Biochem. J., 185,* 139 (1980).
13 M. T. Wilson, P. Jensen, R. Aasa, B. G. Malmström and T. Vänngård, *Biochem. J., 203,* 483 (1982).
14 M. T. Wilson, J. Petersen, E. Antonini, M. Brunori, A. Colosimo and J. Wyman, *Proc. Natl. Acad. Sci. U.S.A., 78,* 7115 (1981).

I4

The Metal Centers of Cytochrome *c* Oxidase: Structure and Function

SUNNEY I. CHAN*, DAVID F. BLAIR, CRAIG T. MARTIN, HSIN WANG, JEFF GELLES, J. E. MORGAN, STEVE WITT, R. R. BIRGE, TOM H. STEVENS and GARY W. BRUDVIG

Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, Calif. 91125, U.S.A.

Considerable progress has been made in recent years on the structure of the metal centers in cytochrome *c* oxidase. Most of these studies have naturally focused on the ligands of the metal center as they play a prominent role in electron transfer, oxygen reduction and possibly also energy conservation. The most unambiguous structural information on the ligands has emerged from EPR/ENDOR studies, particularly when these studies are undertaken in conjunction with isotopically substituted cytochrome *c* oxidases prepared by incorporating selectively isotopically substituted amino acids into the protein via biosynthetic procedures. These results will be reviewed.

Good progress has also been made towards elucidating the mechanism of dioxygen reduction. Preliminary evidence for a mechanism involving both a peroxo and a ferryl intermediate will be presented. The possible structural differences at the dioxygen reduction site between the resting oxidized enzyme and the pulsed enzyme will also be discussed.

While impressive progress has been made toward understanding the structure of the metal centers of cytochrome oxidase and their roles in dioxygen reduction, comparatively little is known about the mechanisms by which the free energy of this reaction is conserved in the form of a transmembrane electrochemical potential gradient. It appears likely that the protons consumed in the reduction of dioxygen to water are derived from the matrix side of the mitochondrial membrane. Since the electrons used in this reaction originate from the intermembrane space of the mitochondrion, a transmembrane electrochemical potential gradient will be generated. However, the spatial dispositions of the metal centers in the membrane profile (or along the transmembrane electrochemical potential profile) are not sufficiently known

to allow one to pinpoint the electron transfer step(s) which will lead to energy conservation by this mechanism. The available data which bear upon this question will be discussed briefly and new experimental approaches will be suggested.

Another means of conserving the available energy is pumping protons from the mitochondrial matrix to the intermembrane space. Evidence that the enzyme is, in fact, a proton pump will be reviewed, as will some of the current models for the mechanism of this pumping. The relative merits of each of the metal centers as the site of proton pumping will be considered. The implications of electron transfer rate theory for the mechanism of proton pumping and the positioning of the metal sites in the transmembrane electrochemical potential profile will be explored. It is suggested that Cu_A is the most suitable candidate for the proton pump; available evidence for this hypothesis will be presented.

The cytochrome oxidase-catalyzed dioxygen reduction reaction will probably not release energy in uniform increments. Thus, it might be expected that some electron-transfer steps will not lead to proton pumping, particularly under conditions of high membrane potential. This possibility has not been adequately appreciated by investigators who attempt to assign fixed proton/electron stoichiometrics to the cytochrome oxidase reaction. This question will be discussed with reference to available information on the thermodynamics of this reaction. Simple mechanisms by which the enzyme might adapt to a changing membrane potential will be described.

I5

Spin Coupling Models for Cytochrome Oxidase

CHRISTOPHER A. REED* and WILLIAM F. SCHOLZ

Chemistry Department, University of Southern California, Los Angeles, Calif. 90089, U.S.A.

GEORGE LANG

Physics Department, Pennsylvania State University, University Park, Penn. 16802, U.S.A.

Spin coupling between high-spin ferric heme a_3 and cupric Cu_B is the most popular explanation for the EPR silent active site of resting state cytochrome oxidase [1]. In this paper we examine how synthetic model compounds with spin coupling between S = 5/2 ferric porphyrins and adjacents S = 1/2 centers support this hypothesis.

Communications from a number of different laboratories chronicle the progress which is being made in the synthesis of cytochrome oxidase models having iron(III) porphyrins which are chemically linked to adjacent copper(II) centers [2–6]. While we await the evolution of this model compound approach and the full structural and magnetic characterization of these materials it is instructive to consider the properties of two magnetically interesting iron(III) porphyrins which might appear at first glance not to be particularly relevant to the iron–copper spin coupling problem. The π-radical cations of iron(III) porphyrins are, however, the best characterized of the very few known examples of S = 5/2 heme groups which are spin coupled to nearby S = 1/2 centers.

The two complexes $[FeCl(TPP\cdot)]^+$ and $Fe(OClO_3)_2(TPP\cdot)$ have been characterized unambiguously as high-spin iron(III) complexes of the tetraphenylporphyrin π-cation radical (TPP·) [7–9]. Both are EPR silent. However, they are magnetically quite distinct and represent two extremes of magnetic interaction between the S = 5/2 iron(III) centers and the S = 1/2 ligand. The first complex, $[FeCl(TPP\cdot)]^+$, has a linear 4–300 K Curie magnetic susceptibility plot with values that identify it as an S = 2 system (μ_{eff}^{300K} = 5.1 BM). To our knowledge this is the only known example of strong antiferromagnetism ($|-J| \gg 200$ cm^{-1}) between S = 5/2 and S = 1/2 centers and as such, it provides a conceptual spin model for the widely accepted, albeit unproven, S = 2 model for the heme a_3/Cu_B site of oxidase. The second complex, $Fe(OClO_3)_2(TPP\cdot)$, also has a linear Curie plot but its magnetic moment (μ_{eff}^{300K} = 6.1 BM) requires a higher spin multiplicity. The two possibilities are an S = 3 ferromagnet ($+J \gg 200$ cm^{-1}) or an independent S = 5/2, S = 1/2 spin system. Magnetic measurements and Mössbauer data favor the latter assignment [8]. This is intriguing from many points of view not the least of which is the possibility that this system has no detectable spin coupling in a magnetic susceptibility experiment (4–300 K) and yet is EPR silent under normal spectrometer conditions (10 K).

Mössbauer effect measurements on these complexes are also of interest in considering spin-coupling models for oxidase. Both complexes show zero field Mössbauer parameters for the isomer shift (δ) and quadruple splitting (ΔE_Q) which are quite typical of high-spin ferric porphyrins [7–9]. However, Mössbauer spectra run in applied magnetic fields reveal differences ascribable to the effects of spin-coupling in $FeCl(TPP\cdot)^+$ [8]. These results provide but one demonstration of a hypothesis which probably has general validity, namely, isomer shift and quadrupole splitting parameters are reliably diagnostic of spin state and oxidation state in spite of possible spin coupling to adjacent paramagnetic centers. It is linewidth information and data collected in the presence of applied magnetic fields which give clues to the presence of spin coupling although its nature can be difficult to decipher. These observations support the spin and oxidation state assignments made recently for heme a_3 from Mössbauer effect studies with bacterial oxidase [10] and at the same time lead to