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

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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

some uncertainty in reconciling the low-spin ferric-like Mössbauer parameters of one model complex [5] with its proposed spin state and structure.

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I6

Is Cytochrome aa_3 From *Thermus Thermophilus* a Single Subunit Oxidase?

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A reliable procedure has been developed for the purification of the cytochrome c_1aa_3 complex from the plasma membrane of *T. thermophilus.* The ratios heme C:heme A:Fe:Cu were found to be 1:2:3:2 confirming previous results, however, the molecular weight was found to be ~92,000 rather than the ~200,000 reported earlier [1]. Polyacrylamide gel electrophoresis under strongly denaturing conditions and high performance reverse phase liquid chromatography showed that cytochrome c_1aa_3 is composed of only two subunits in 1:1 ratio. Both polypeptides have blocked N-termini. The smaller subunit (~33,000) binds heme *c* and presumably no other metals. The larger subunit (~55,000) is thus thought to contain the elements of cytochrome aa_3 and therefore must be considered a single subunit cytochrome oxidase.

The bacterial cytochrome c_1aa_3 has been compared with beef heart cytochrome oxidase with a number of techniques including optical, EPR [1], Raman, MCD, and Mössbauer [2] spectroscopies. These experiments establish that the fundamental chemical properties of the redox centers are substantially similar in these two proteins.

Cytochrome c_{552} (from *Thermus*), horse heart cytochrome *c*, and tetramethylphenylenediamine greatly stimulate the ascorbate oxidase activity of cytochrome c_1aa_3. This enhancement is characterized by a 'high affinity' component which results in only a small velocity increase and a 'low affinity' component which gives a large velocity increase. Very similar behavior has been previously observed with mammalian cytochrome oxidase [3].

Preliminary experiments show that vesicularized c_1aa_3 is capable of proton pumping.

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I7

Aspects of the Chemistry of the Two Heme Centers of Cytochrome Oxidase

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Derivatives of heme *a* have been examined by optical, MCD and EPR spectroscopy [1]. Five- and six-coordinate high-spin ferric species exhibit optical spectra recently classified as 'Type a' by Quinn *et al.* [2] while a low-spin bis-imidazole ferric derivative exhibits a 'Type b' spectrum. On reduction the visible spectrum of the low-spin derivative intensifies markedly and exhibits a single maximum at 589 nm; the visible spectrum of the high-spin species changes shape but the intensity is only slightly changed. The ferric high-spin compounds exhibit a transition in the near-infrared which has absorbance and MCD characteristics similar to the 655 nm band [3] of the resting enzyme.

Composite spectra obtained by the addition of the individual spectra of the ferric high- and low-spin models and of the ferrous high- and low-spin models reproduce the essential features of the spectra of oxidized and reduced enzyme, respectively. The relative contributions of the high- and low-spin derivatives to the spectral changes at 589 nm produced by