than 2 electrons to the redox sites in the enzyme. In a small fraction (approx. 20%) of the oxidase molecules, however, 3 electron equivalents can enter the enzyme. This is evidenced by the appearance of the low-spin Fe^{3+} HCN-cytochrome a_3 signal at $g = 3.55$, indicating that Cu_B must be reduced. Thus, only the fraction of the molecules that has accepted 3 electron equivalents shows a cyanide EPR signal whereas in the remaining 80% of the molecules no cyanide signals could be found. Such heterogeneities due to mixtures of different enzyme forms have been widely reported. The absence of cyanide signals can be explained if cyanide binds as a bridging ligand between the antiferromagnetically coupled cytochrome a_3 and Cu_B sites.

A mechanism with open and closed enzyme forms could have a functional significance in facilitating 2-electron reduction of O_2, bound at the a_3 - Cu_B site, as a way of avoiding the formation of toxic superoxide ions. Alternatively, such conformational changes could be part of the energy transduction system, *e.g.* the mechanical part of a proton pump.

Note: A full description of the experiments can be found in *Biochem. J.* (1984), in press.

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REDOX PATHWAYS IN *c*-TYPE CYTOCHROMES. POSSIBLE ROLE OF THE BRIDGING SULFUR

Rates of reduction of various classes of heme proteins by small organic reductants have been correlated with the degree of exposure of the heme to solvent [1]. For the *c*-type cytochromes (cyt), the heme edge about ring II is exposed in a cleft. That this exposed heme edge may be important for electron transfer to and from the heme iron has been considered by many researchers whose suggestions have generally focussed on the peripheral aromatic carbons of the porphyrin. Examination of the van der Waals surfaces of the *c*-type cytochromes of known crystal structure reveals [2] that the sulfur of the thioether bridge that covalently links ring II to the protein (the sulfur of Cys-17 of cyt *c*) protrudes from the heme edge and the protein surface. If delocalization of ring π and iron d orbitals occurs onto this sulfur, then it effectively extends electron density from the aromatic edge of the heme by ~2.4 Å. For self-exchange reactions this would decrease the distance over which the electron has to tunnel. For reactions with small molecule reductants and oxidants, the sulfur could provide an accessible site on the protein surface.

The possibility that such delocalization may occur was examined theoretically with charge-iterative extended Hückel calculations. Low-spin Fe(III) and Fe(II) porphins, substituted at the 2- and 4-positions with $C(CH_3)HSCH_3$ groups, and ligated to imidazole and dimethylsulfide were calculated. In the Fe(III) calculations, the acceptor t_{2g} orbital of the iron (either d_{xz} or d_{yz}) was found to be delocalized ~25-30% onto the porphyrin macrocycle, including the bridging sulfurs. The amount of unpaired spin density calculated for a sulfur, 1-4%, is comparable to values obtained for individual aromatic ring carbons. In the Fe(II) case, the t_{2g} orbitals are much less delocalized. However, the porphyrin $a_{1u}(\pi)$ orbital contains a large contribution from the bridging sulfur. Thus, since both porphyrin π and Fe(III) t_{2g} orbitals are predicted to delocalize out to the bridging cysteinyl sulfurs, the calculations suggest that the exposed sulfur off ring II may indeed facilitate electron transfer.

ACKNOWLEDGEMENTS

This work was supported by the Division of Chemical Sciences, U.S. Department of Energy, Washington, D.C., under Contract No. DE-AC02-76CH00016.

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PS2.22 — MO

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TIME-RESOLVED RESONANCE RAMAN STUDY OF THE OXIDATION OF CYTOCHROME OXIDASE BY DIOXYGEN

Cytochrome oxidase catalyzes the rapid and efficient reduction of dioxygen to water. The sequence of events which occurs in this mechanistically complex reaction has been difficult to elucidate owing to the rapid rates of O_2 binding ($\sim 10^8$ M^{-1} s^{-1}) and electron transfer ($\sim 10^5$-10^3 s^{-1}) [1]. To overcome this kinetic obstacle, flash photolysis of the carbon monoxide complex of the reduced protein has been used to initiate the oxygen reduction reaction. This «flow-flash» approach was pioneered by GIBSON and GREENWOOD for room temperature spectrophotometry [2], and adapted by CHANCE and coworkers to low temperature optical, EPR and EXAFS spectroscopies [3]. Although these approaches have clarified the kinetics of the reaction [4], basic structural questions concerning the reaction sequence remain unresolved. Owing to this situation and to uncertainties in extrapolating the low temperature results to room temperature [5], we have combined flow-flash and time-resolved resonance Raman techniques to study the cytochrome oxidase/oxygen reaction at room temperature.

Cytochrome oxidase was prepared from beef heart [6a] and, following anaerobic reduction, combined with carbon monoxide. The solution was loaded into one syringe and oxygenated buffer into a second syringe. These were mounted on a syringe pump and connected to a Gibson-type, four-jet tangential mixer [7]. The mixed solution flowed into the Raman cuvette upon which the Q-switched pulses (10 ns duration) of two Quanta Ray Nd:YAG lasers were focussed. The pulse from the first laser was the 532 nm Nd:YAG second harmonic. Its energy was sufficient to photolyze the CO cytochrome oxidase complex and initiate the O_2 reduction reaction. Raman scattered light from the second, time delayed pulse at 416 nm was dispersed and detected by an EG&G/PARC 1420 diode array detector [8]. The delay times between the green photodissociation and violet probe pulses were variable from 10 ns to approximately 1 ms.

Fig. 1 shows Raman spectra of cytochrome oxidase at various times after initiation of the reoxi-

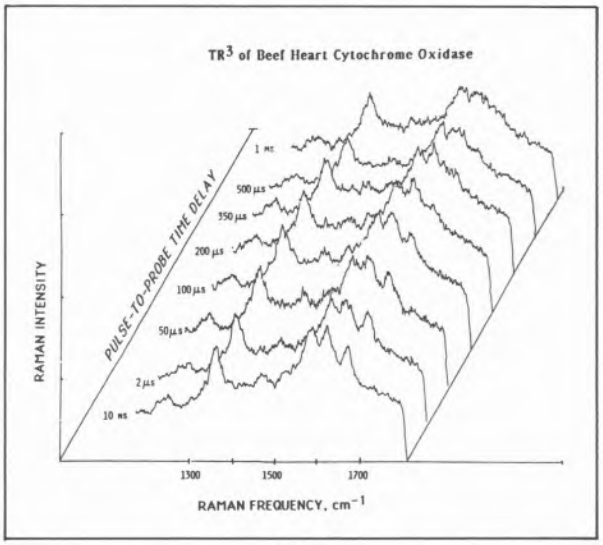

Fig. 1
Raman spectra of cytochrome oxidase at the indicated times following initiation of the oxygen reduction reaction at room temperature

dation reaction. The 10 ns spectrum is that of the photodissociation product of the reduced carbon monoxy enzyme. The oxidation state marker ($\bar{\nu}_4$, 1355 cm^{-1}) and the a_3^{2+} formyl stretching frequency ($\bar{\nu}_{C=O}$, 1666 cm^{-1}) [6] are well-resolved