

tion [2]) are greater than millimolar which should give rise to bimolecular kinetics. This raises the possibility of a second C binding site on CCP which is further removed from the peroxidase heme and therefore does not give rise to spectral perturbations. Alternatively, the slow process could be a rate-limiting conformation change of the peroxidase followed by bimolecular electron transfer.

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REFERENCES

- [1] T. YONETANI, *The Enzymes*, **8**, 345 (1976), and references therein.
- [2] a) J.E. ERMAN, L.B. VITELLO, *J. Biol. Chem.*, **255**, 6224 (1980).
b) C.H. KANG, S. FERGUSON-MILLER, E. MARGOLIASH, *J. Biol. Chem.*, **252**, 919 (1977).
- [3] M.J. POTASEK, *Science*, **201**, 151 (1978).
- [4] $E^\circ (C^{III/II}) = 0.26$ V from
G.R. MOORE, D.E. HARRIS, F.A. LEITCH, G.W. PETTIGREW, *Biochim. Biophys. Acta*, **764**, 331 (1984);
 $E^\circ (CCP^{III/II}) = -0.20$ V from
C.W. CONROY, P. TYMA, P.H. DAUM, J.E. ERMAN, *Biochim. Biophys. Acta*, **537**, 62 (1978).
- [5] T.C. CHEUNG, Y. HO, A.M. ENGLISH, J.A. KORNBLATT, G. MCLENDON, K. TAYLOR, J.R. MILLER, in preparation.
- [6] a) T.L. POULOS, S.J. FREER, R.A. ALDEN, S.L. EDWARDS, U. SKOGLAND, K. TAKIO, B. ERICKSSON, N. XUONG, T. YONETANI, J. KRAUT, *J. Biol. Chem.*, **255**, 575 (1980);
b) R. SWANSON, B.L. TRUS, N. MANDEL, G. MANDEL, O.B. KALLAI, R.E. DICKERSON, *J. Biol. Chem.*, **252**, 759 (1977).
- [7] T.L. POULOS, J. KRAUT, *J. Biol. Chem.*, **255**, 10322 (1980).
- [8] C.E. NELSON, E.V. SITZMAN, C.H. KANG, E. MARGOLIASH, *Anal. Biochem.*, **83**, 622 (1977).
- [9] B. WARD, C.K. CHANG, *Photochem. Photobiol.*, **35**, 757 (1982).



PS2.20 — TU

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CYANIDE-INHIBITION STUDIES ON CYTOCHROME *c* OXIDASE USING RAPID-FREEZE/EPR

It has been known for a long time that cyanide inhibition of partially reduced cytochrome *c* oxidase occurs with a higher rate than with the oxidized or reduced enzyme. This partial reduction has been suggested to cause a conformational change, leading to a transition to a more active enzyme form and thus rendering its cytochrome a_3 site more available to cyanide binding. An enzyme model with only 2 conformational states, *resting* and the more active *pulsed* form, was found to be inadequate and the model had to be extended to include an *open* and a *closed* conformational state of both the pulsed and the resting form. A partial reduction of the 4 redox centers in the oxidase (cytochrome *a* and a_3 , Cu_A and Cu_B) was proposed to be the trigger mechanism for the transition from closed to open conformation, enabling fast cyanide binding.

To investigate the number of electrons necessary to trigger this conformational change, a rapid freeze/EPR investigation was performed. The data shows that the majority of the enzyme molecules in both resting and pulsed state are rapidly inhibited by cyanide when cytochrome *a* and Cu_A have been reduced and this prevents entry of more

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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.



PS2.21 — TU

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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 $\text{C}(\text{CH}_3)\text{HSCH}_3$ 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.

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REFERENCES

- [1] T.E. MEYER, C.T. PRZYSIECKI, J.A. WATKING, A. BHATTACHARYA, R.P. SIMONSEN, M.A. CUSANOVICH, G. TOLLIN, *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 6740 (1983).
- [2] G. TOLLIN, M.A. CUSANOVICH, private communication.