either in the presence or in the absence of NADH, the way by which the oxidized methionine takes back the electron is different in the two cases.

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PS2.2 — TU

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THE HEME ENVIRONMENT OF PEROXIDASES: ^{15}N NMR OF BOUND $C^{15}N^-$

Cyanide ion potentially can provide a useful NMR spectroscopic probe of the heme environment in ferric hemoproteins. Both carbon-13 and nitrogen-15 cyanide signals have been detected for model cyanoiron(III) porphyrin complexes [1-3]. Nitrogen-15 signals suffer less paramagnetic broadening, and accordingly the resonances have also been observed in a far downfield region in hemoglobin, myoglobin, and cytochrome *c* [3].

Coordinated cyanide ^{15}N chemical shifts for peroxidases are reported here for the first time. Chemical shift values are highly distinctive for these enzymes as compared with those for other hemoproteins. Table I presents a summary of ^{15}N chemical shift data for ferric hemoproteins. The striking upfield shift for peroxidase cyanide signals appears to be directed by both strong distal hydrogen bonding effects and by presence of a polar amino acid ligand *trans* to the cyanide residue.

Table I
Nitrogen-15 Chemical Shift Values

Compound	CN^- Chemical Shift (ppm)[a]
Myoglobin(CN)	948[b]
Hemoglobin(CN)	975, 1047[b]
Cytochrome *c*(CN)	847[b]
Horseradish Peroxidase(CN)	578
Lactoperoxidase(CN)	423
Chloroperoxidase(CN)	412
Cytochrome *c* Hemopeptide-11(CN)	749
ProtDMEFe(CN)(Imidazole)	1015[c]
ProtDMEFe(CN)(Imidazolate)	738[c]

a) Rel. to NO_3^-, 25°C, pH 7 to 8. b) Ref. [3]. c) DMSO solvent.

A *trans* ligand influence is seen for the model cyano-imidazole iron(III) protoporphyrin dimethyl ester complex upon deprotonation of the imidazole ligand. An upfield ^{15}N shift of 277 ppm follows generation of the imidazolate species. Putative ligation of a deprotonated cysteinyl sulfur residue in chloroperoxidase can thus be invoked to explain in part the relatively far upfield ^{15}N cyanide resonance in this enzyme. Partial imidazolate character (through proximal imidazole hydrogen bonding) in horseradish peroxidase likewise may influence the upfield character of the coordinated cyanide ion in this enzyme. The relatively high-field ^{15}N signal for lactoperoxidase could be taken to imply presence of a charged *trans* amino acid residue. Although histidyl imidazolate coordination cannot be dismissed for the iron(III) state, separate ^{13}C NMR experiments using CO as a probe are consistent with *trans* imidazole coordination in the carbonmonoxy state.

On the basis of model studies, distal hydrogen bonding of the bound cyanide ion in peroxidases can also be invoked as contributing to the upfield bias for ^{15}N NMR signals in these enzymes. The role of protic solvents in directing an upfield bias for cyanide signals of model compounds has been noted previously [3]. We have also demonstrated that addition of a non-coordinating imidazole to a benzene solution of dicyanoiron(III) protoporphyrin dimethyl ester effects an upfield shift of 82 ppm presumably through Im-H---N-C-Fe hydro-

gen bonding. An upfield shift of 98 ppm for the cytochrome *c* hemopeptide resonance as compared with that of cytochrome *c* may reflect the influence of exposure of the distal site of the model compound to aqueous (hydrogen-bonding) solvent.
The striking upfield bias of cyanide resonances for peroxidases as compared with other hemoproteins must parallel the reactivity and heme pocket differences among the biomolecules. Neither hydrogen bonding nor *trans* ligand effects alone provide for exact model compound simulation of the ^{15}N resonance values in peroxidases. Hence both contributions must dictate the level of unpaired spin delocalization at the cyano nitrogen atom. Likewise, these effects may be responsible for the very efficient heterolytic cleavage of peroxides by peroxidase enzymes. The unique ^{15}N chemical shifts for peroxidases are supportive of the concept that a distal hydrogen bonding network [4] and perhaps a polar, basic *trans* ligand are essential for O-O bond activation by peroxidases and by cytochrome P-450.

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PS2.3 — TH

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ELEMENTS OF THE PROPOSED PEROXIDASE MECHANISM ELUCIDATED FROM NMR AND IR STUDIES OF CYTOCHROME *c* PEROXIDASE FORMS

INTRODUCTION

Cytochrome *c* peroxidase (EC 1.11.1.5, ferricytochrome *c*: oxidoreductase; CcP) is a ferriheme enzyme typical of the class of peroxidases. Isolated from baker's yeast, CcP's physiological role is thought to be catalysis of the hydrogen peroxide oxidation of ferrocytochrome *c* [1,2]. The catalytic cycle of CcP involves heme iron in the +4 oxidation state (oxidized intermediates I,II), and recently the precise steps in the CcP catalytic mechanism have come under intense study [3-6]. The result has been the proposal of a specific mechanism for CcP [6]. In this work we present the results of our recent NMR and infrared spectroscopy studies of ferric and ferrous CcP forms that elucidate specific parts of the Poulos-Kraut mechanism [6].

EXPERIMENTAL

Cytochrome *c* peroxidase was isolated and purified as previously described [7]. Proton Nuclear Magnetic Resonance and infrared spectroscopies were performed as previously described [8,9].

RESULTS AND DISCUSSION

The proposed catalytic mechanism of cytochrome *c* peroxidase (Poulos-Kraut mechanism) involves a