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

JAMES D. SATTERLEE
Department of Chemistry
University of New Mexico
Albuquerque, New Mexico 87131
U.S.A.
JAMES E. ERMAN
Department of Chemistry
Northern Illinois University
DeKalb, Illinois 60115
U.S.A.

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

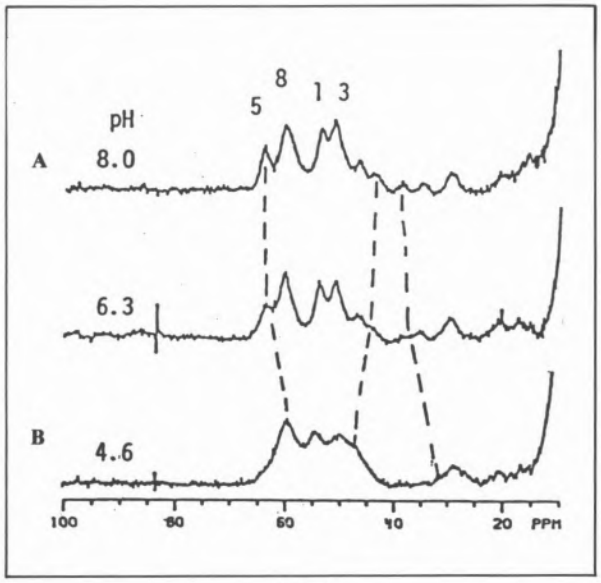

Fig. 1
pH Dependence of CcP-F Hyperfine Proton Resonances

«catalytic triad» of distal amino acid residues that participate in the decomposition of heme coordinated hydrogen peroxide [5,6]. These amino acids, Arg-48, Trp-51, His-52, may function as hydrogen bond donors in fulfilling their function. Elsewhere, we have detected the presence of hydrogen bonding to a heme bound ligand [8], indicating the validity of such a role for the distal amino acids. We show here the effect of pH upon the visible and infrared spectra of CcP-CO and determine the pK of the hydrogen bonded proton as ~ 8.4. Another aspect of CcP function is the pK at 5.5 that regulates the native enzyme's reactivity and ligand-binding properties. Recently it has been shown that the X-ray difference maps of CcP *vs.* CcP-F indicate a specific movement of Arg-48 to a position that enables it to hydrogen bond to heme coordinated fluoride ion [10,11]. When we compare these results to our recent NMR data on CcP-F we find that three proton hyperfine resonances (a methyl group and two single protons, Fig. 1A-B) exhibit specific titrations with pK values ~ 5.5. One of these resonances is an assigned heme methyl group at position 5 of the heme ring [12]. The specificity of this effect indicates that we are probably observing a pH-dependent movement of Arg-48 within the heme pocket. We would like to draw attention to the similarity of the CcP-F pH dependence with that reported for the native enzyme [9]. It may well be that both pH dependences relate to positioning the Arg-48 so that it may participate in the hydrogen peroxide decomposition [5].

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PS2.4 — MO

DAVID MYERS
GRAHAM PALMER
Department of Biochemistry
Rice University
P.O. Box 1892, Houston, TX 77251
U.S.A.

MCD STUDIES ON THE HEME AND TRYPTOPHAN COMPONENTS OF CYTOCHROME *c* PEROXIDASE

We have measured the magnetic circular dichroism of cytochrome *c* peroxidase (CCP) and some of its derivatives from 250-350 nm. Comparison of the changes observed on conversion to the catalytic intermediate (CCP-I) with spectra obtained from horseradish peroxidase and its derivatives and model compounds of protoheme leads us to the conclusion that the observed changes in the MCD spectra reflect conversion of the heme to the ferryl state. No evidence was found for modification of tryptophan in CCP-I.

PS2.5 — TU

ELKA V. ELENKOVA
MASANORI SONO
JOHN H. DAWSON
Department of Chemistry
University of South Carolina
Columbia, SC 29208
U.S.A.
ANN M. ENGLISH
Department of Chemistry
Concordia University
Montreal H3G 1M8
Canada

MAGNETIC CIRCULAR DICHROISM STUDIES OF CYTOCHROME *c* PEROXIDASE AND ITS LIGAND COMPLEXES

INTRODUCTION

Yeast cytochrome *c* peroxidase (CCP) is a soluble heme protein, located in the mitochondrial intermembrane space, that catalyzes the two-electron reduction of hydroperoxides by ferrocytochrome *c* in the following reaction:

$$2\ \text{cyt}\ c(Fe^{2+}) + ROOH + 2H^{+} \rightarrow 2\ \text{cyt}\ c(Fe^{3+}) + ROH + H_2O$$

CCP contains a noncovalently bound protoheme IX prosthetic group and has a known amino acid sequence. The crystal structure of CCP has recently been published [1]. Despite the above information, questions still remain about the relationship between the physical structure of CCP and its catalytic properties. We have used magnetic circular dichroism (MCD) spectroscopy to probe the electronic, and therefore indirectly the physical, structure of native ferric and ferrous CCP and its complexes with CN^-, N_3^-, F^-, CO, NO and of CCP-Compound I. In order to provide a basis for comparison, the MCD data on CCP, reported here for the first time, have been compared to analogous data from other imidazole-ligated heme proteins such as myoglobin (Mb) and horseradish peroxidase (HRP).