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(12) This material, which had λ_{max} (pH 8) 258 nm, was homogeneous on LC (Partisil 10 SAX, 0.5 M potassium phosphate buffer (pH 3.35)) and paper electrophoresis (0.1 M sodium acetate buffer (pH 4.0)) and free from the isomeric adenylyl-(3′→5′)-adenylyl-(3′→5′)-adenosine; it was completely digested to adenosine 5′-phosphate (two parts) and adenosine (one part) in the presence of *Crotalus adamanteus* snake venom phosphodiesterase and was resistant to calf spleen phosphodiesterase promoted hydrolysis.
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(14) This corresponds to the previously reported procedure (A. F. Cook, M. J. Holman, and A. L. Nussbaum, *J. Am. Chem. Soc.*, **91**, 1522 (1969)) for the conversion of 3′-*O*-acetylthymidine 5′-*S*-ethylphosphorothioate into the corresponding 5′-triphosphate. We have similarly prepared[15] adenosine 5′-triphosphate from 2′,3′-*O*-methoxymethylene-6-*N*-benzoyladenosine 5′-*S*-methylphosphorothioate.
(15) S. S. Jones and C. B. Reese, unpublished observations.
(16) Despite the precautions taken, it was probably impossible to remove the last traces of water from the reaction mixture and the pyrophosphate used was shown by ^{31}P NMR spectroscopy to contain 27% orthophosphate. Thus the presence of **11c** and **11d** in the products was not unexpected.

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Models for the Reduced States of Cytochrome P-450 and Chloroperoxydase. Structures of a Pentacoordinate High-Spin Iron(II) Mercaptide Mesoporphyrin Derivative and Its Carbonyl Adduct

Sir:

Substantial clarification of the nature of the cytochrome P-450 oxygenase reactions has been obtained recently by isolation of the soluble cytochrome (P-450 cam) from *Pseudomonas putida* grown on camphor.[1] Assembly in vitro of the enzyme system has led to the reaction sequence shown in Scheme I.

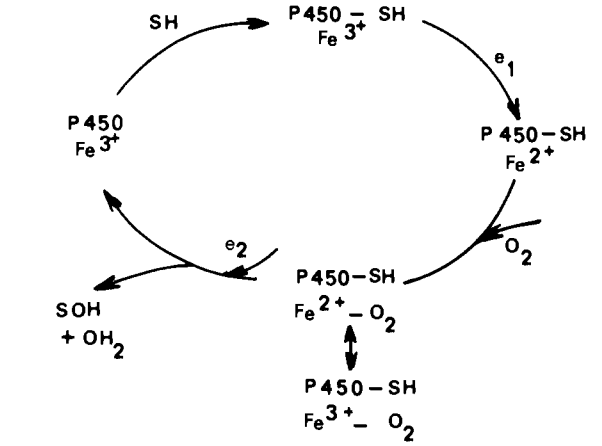
Chloroperoxydase was detected in the mold *Caldariomyces fumago*.[2] In the reduced state it shows spectral properties very similar to those of cytochrome P-450. The CO adducts of both enzymes in the reduced state exhibit hyperporphyrin-type spectra with the Soret band at 450 nm.[3]

Although the P-450 reaction sequence has been established, comparatively little is known concerning the structural details of the active site of these enzymes, especially in the reduced states. Ferric and ferrous mercaptide porphyrin complexes have been prepared exhibiting spectroscopic properties similar to those of several reaction states of both enzymes.[4-8] However, only the structural properties of ferric model complexes have been established at this time.[7,8] EXAFS studies of a microsomal P-450 enzyme and chloroperoxydase from the fungus *Caldariomyces fumago* in their ferric forms are also consistent with a mercaptide sulfur as axial ligand.[9]

We now report the synthesis and X-ray studies of two iron(II) porphyrin derivatives $[Na\subset 222][C_2H_5SFeTPP]\cdot 2C_6H_5Cl$ (I) and $[Na\subset 221]_2[SC_2H_5FeTTP(CO)]\cdot SC_2H_5\cdot 1.5C_6H_6$ (II), presenting spectroscopic features similar to those of the ferrous and ferrous carbonyl states of P-450 and chloroperoxydase. (TPP and TTP are the dianions of tetraphenylporphyrin and tetra-*p*-tolylporphyrin, respectively.)

To facilitate crystallization, the syntheses were conducted using the preparative conditions previously adopted by Colman et al.[6] (C_6H_6 or C_6H_5Cl solutions) and substituting crown ethers by macrocyclic diazapolyoxa cryptands (221 or 222) to enhance the solubility of the mercaptide in these media. A tenfold mercaptide excess is required to achieve successful synthesis. Solvents must be freshly distilled and carefully degassed before use. Crystals were obtained by slow pentane diffusion into the solvents selected for the synthesis.[10]

Scheme I



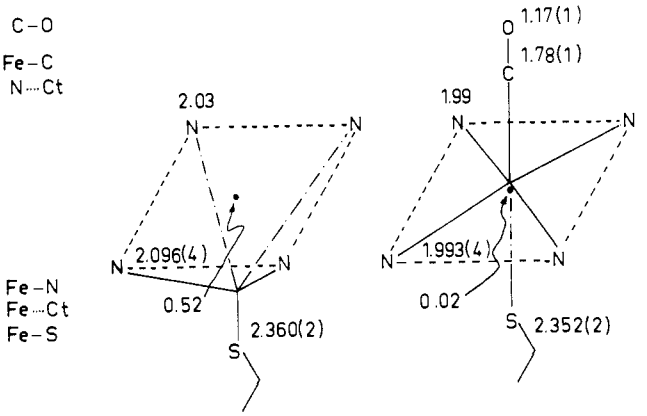


Figure 1. Summary of the main distances (ångstroms) and coordination spheres of iron in $[C_2H_5SFeTPP]^-$ (I) and $[C_2H_5SFeTTP(CO)]^-$ (II).

The coordination spheres of iron(II) in I and II are summarized in Figure 1. In I the iron atom is pentacoordinated and the average Fe–N_p distance of 2.096 ± 8 Å is slightly longer than those present in the two structures of high-spin iron(II) porphyrin complexes known so far, Fe(TTP)-2-Me-Im (III) (2.086 ± 6 Å) and Fe(TpivPP)-2-Me-Im (IV) (2.072 ± 4 Å).[11,12] Consequently the displacement of the iron atom with respect to the mean plane of the four nitrogen N_p is considerably larger (0.52 Å); the displacement of the metal atom with respect to the mean plane of the 24-atom core is 0.62 Å. In III and IV the displacements of the metal atoms relative to the mean plane of the four porphyrinato nitrogen atoms are 0.42 and 0.399 Å, respectively. In addition the doming of the porphyrinato skeleton is smaller in I (0.10 Å) than in III (0.15 Å) but larger than in IV (0.03 Å). Considerably more buckling is present in I than in III but less than in IV; the mean displacement from the 24-atom core mean plane is 0.105 Å. Some of the doming may be a result of crystal-packing forces, but the structure of I shows clearly more doming than five-coordinated metalloporphyrins in general; for instance, in Fe(PP IX DME)$SC_6H_4NO_2$ the doming parameter is 0.014 Å. This large doming explains at least in part (i) the predominant formation of the pentacoordinate species with a mercaptide ligand; (ii) the low CO affinity of this pentacoordinate complex (vide infra). No iron(II)–mercaptide sulfur bond distances are known when this group is engaged in a porphyrin ring. As expected this bond length of 2.360 (2) Å is somewhat longer than that present in the pentacoordinate iron(III) porphyrin complex Fe(PP IX DME)SC_6H_4-*p*-NO_2 (2.324 (2) Å).[7]

The involvement of a pentacoordinate high-spin ($S = 2$) ferrous mercaptide heme species in the P-450 cycle has previously been postulated.[13] Mössbauer studies of the reduced protein have confirmed the presence of a pentacoordinate

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species.[14] The present work constitutes the first structural indications of what could be the active site in the reduced pentacoordinate state of P-450. Indeed, I contains a high-spin ($S = 2$) pentacoordinate ferrous mercaptide porphyrin species. Solutions of I in chlorobenzene present a magnetic moment of 4.8 ± 0.1 μ_B (NMR) indicating that the pentacoordinate high-spin iron(II) complex is also predominant in that medium.[6] The visible spectrum presents bands at 422 (5.13), 530 (3.87), 580 (3.73), 630 (3.60) nm in chlorobenzene. These solutions quickly pick up CO to give rise to a compound displaying a hyper-porphyrin-type spectrum with bands at 386 (4.94), 458 (4.96), 565 (3.63), and 615 (3.63) nm.[15] CO absorption can be followed in the infrared where a new absorption appears at 1920 cm^{-1}. This reaction is completely reversible and the spectrum of I is regenerated when the UV cell is purged with argon. It is noteworthy that CO uptake also occurs reversibly in the solid state (ν_{CO} 1930 cm^{-1}).

The carbonyl adduct has been crystallized as the TTP complex (II) and an X-ray analysis at 75 K has confirmed the presence of CO at the sixth coordination site. II is diamagnetic in solution. The porphyrin ring is planar. At the present stage of refinement, the average Fe-N_p bond distance is 1.993 (4) Å and a general contraction of the porphyrinato core attributable to a high-spin to low-spin transition of the iron atom takes place (0.04 Å). The displacement of the iron atom with respect to the mean plane of the four nitrogen atoms (0.02 Å) is within experimental error. The Fe-S bond distance of 2.352 (2) Å is not significantly different from that of 2.360 (2) found in I.[16] Fe-C and C-O bond lengths (1.78 (1) and 1.17 (1) Å) are not significantly different from those present in Fe(TTP)(py)(CO).[17]

Mossbauer spectral studies of I and II and X-ray studies of other iron mercaptide porphyrin complexes are presently underway.

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(10) (a) Preparation of compound I was carried out in C_6H_5Cl using FeTPP and [Na⊂222]SC_2H_5. It crystallizes as a bischlorobenzene solvate in space group $P2_1/c$ with a = 13.238 (4), b = 20.999 (6), c = 25.456 (7) Å; β = 94.85 (5)°; Z = 4. A total of 10 332 reflections were recorded at room temperature using a Philips PW1100 diffractometer with filtered Cu Kα radiation ($\theta/2\theta$ flying step scan); 5627 reflections with $I > 3\sigma(I)$ were included in subsequent calculations. (b) Compound II was prepared in C_6H_6 using Fe(CO)TTP and [Na⊂221]SC_2H_5 under CO atmosphere. It crystallizes as a 1.5C_6H_6 and 1[Na⊂221]SC_2H_5 solvate in space group $P\bar{1}$ with a = 21.255 (15), b = 20.696 (13), c = 11.311 (8) Å; α = 93.03 (3), β = 79.43 (4), γ = 108.21 (3)°; Z = 2. To improve diffraction, data were collected at 75 K on a Picker FACS I diffractometer equipped with a CT-38 Cryogenic Association low-temperature device using Mo Kα radiation. A total of 16 500 reflections were measured in the $\theta/2\theta$ integrating scan mode and 5520 observations with $I > 3\sigma(I)$ were retained and used in the refinement. Structure solution and refinement were performed on a PDP 11/60 computer using the Enraf-Nonius structure determination package. The present $R_1 = (\Sigma||F_o| - |F_c||)/|F_o|$ values are, respectively, 0.087 for I with anisotropic temperature factors for all nonhydrogen atoms and 0.098 for II with anisotropic temperature factors for Fe, S, and Na^+; all other atoms were given isotropic temperature factors.
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(16) The asymmetric unit of II contains one cocrystallized [Na⊂221]SC_2H_5 in addition to that required by stoichiometry. The thiolate anion is free and does not interact with the porphyrinato anion core in any way. Its closest neighbors are methylene groups and oxygen and nitrogen atoms of the [Na⊂221] cations located at distances ranging from 3.7 to 4.5 Å. As a result of the presence of a second [Na⊂221], the crystal packing thus consists of alternating layers of cryptate cations and porphyrinato anions.
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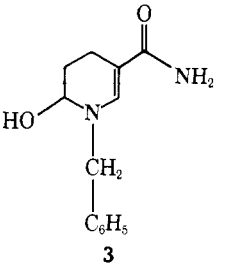
Models for NADH Coenzymes. Evidence for an Electron-Transfer Mechanism Yielding a Radical-Cation Intermediate in the *N*-Benzyldihydronicotinamide–*N*-Benzylnicotinamide Salt Transhydrogenation Reaction

Sir:

The study of the oxidation-reduction reactions of models for nicotinamide coenzymes has provided important information about the mechanism of such processes.[1] We have been investigating the redox mechanism of the *N*-benzyl-1,4-dihydronicotinamide (**1**)–*N*-benzylnicotinamide salt (**2**) transhydrogenation reaction as a model reaction for an NADH dependent redox process[2] (Scheme I). In this communication, we report two major findings: one, the presence of the nicotinamide salt catalyzes the hydration of the dihydronicotinamide, and two, the presence of the nicotinamide salt catalyzes the exchange of the C-4 hydrogen of the dihydronicotinamide with the hydrogen from water. We believe that these observations provide evidence for an electron-transfer mechanism with an intermediate radical–radical-cation pair during the course of transhydrogenation.

An examination of a reaction mixture consisting of 0.05 M *N*-benzyldihydronicotinamide[3] (**1**) and 0.05 M *N*-benzylnicotinamide chloride (**2**) in 0.1 M (pH 8.6) aqueous phosphate by high pressure liquid chromatography (HPLC) revealed the formation of a new product during the course of transhydrogenation at 40 °C. This product was identified as the primary hydration product of *N*-benzyldihydronicotinamide, **3**, by a



3

comparison of spectral and chromatographic properties with those of an authentic sample prepared by the acid-catalyzed hydration of *N*-benzyldihydronicotinamide.[4] The formation of **3** is catalyzed by the presence of 0.1 M phosphate (pH 8.6). If the transhydrogenation is carried out in 0.1 M carbonate or

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