thank Professor Dowd for an advance copy of his paper and for permission to cite it.

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Matthew S. Platz, Jerome A. Berson*
Department of Chemistry, Yale University
New Haven, Connecticut 06520
March 8, 1977

Molecular Dynamics in the Solid State. A Dynamic Model of the Low-Spin Iron(III) to High-Spin Iron(III) Transformation in P450 Enzymes

Sir:

Substrate binding by the cytochrome P450$_{cam}$[1] enzyme isolated from *Pseudomonas putida* has been shown to promote a change of the iron(III) protoporphyrin IX prosthetic group from a low-spin to a high-spin electronic configuration.[2] A change in coordination number from six to five is believed to accompany this transformation.[3] The nature of the axial ligands in the two forms has been the subject of intensive investigation and there is growing evidence that the axial ligand in the five-coordinate ferric form and one of the axial ligands in the six-coordinate ferric form is a deprotonated cysteine (cysS$^-$) residue.[4-6] Although the identity of the second ligand in the six-coordinate form has not been established, histidine, lysine, cysteine (cysSH), and methionine have been suggested as possibilities.

Recently a number of ferrous and ferric complexes have been synthesized that mimic the physical and spectroscopic properties of the active site of P450 at various stages of its catalytic cycle.[5b] Among these is iron(III) tetraphenylporphyrinatobenzenethiolate benzenethiol, FeTPP(C_6H_5S)(C_6H_5SH),[5] for which EPR, Mossbauer, and magnetic susceptibility measurements performed on crystalline samples indicate the presence of both low-spin and high-spin iron(III).[10] At room temperature, this compound is predominantly high spin and, at 4.2 K, primarily low spin. Employing the aforementioned physical techniques, we have found that the populations of the two spin states change continuously as a function of the temperature. Similar spin equilibria have been observed is several complexes of the ferric forms of myoglobin and hemoglobin[11] and in the substrate bound ferric form of P450$_{cam}$.[2,12] A multiple-temperature crystallographic investigation of FeTPP(C_6H_5S)(C_6H_5SH) has been undertaken to examine the structural changes associated with the spin transition. A preliminary phase of this investigation has resulted in the first crystallographic resolution of an equilibrium mixture of spin isomers and has produced a *dynamic* model for the low-spin to high-spin transformation in oxidized P450 enzymes.

Crystallographic data were obtained at 115 K with a Syntex P$\bar{1}$ diffractometer equipped with a locally constructed low-temperature device.[13] Experimental and data-handling techniques were analogous to those described previously.[14] Experimental parameters are summarized in Table I. Standard Patterson, Fourier, and least-squares techniques led to the identification of all nonhydrogen atoms expected from the empirical formula. Full matrix least-squares refinement converged to an acceptable *R* value, but gave large and highly anisotropic thermal parameters for the iron and sulfur atoms as well as several chemically unreasonable interatomic distances. These problems were eventually overcome by refinement of a model which included two alternate configurations for the iron atom and both sulfur-containing ligands. On the basis of independently refined iron and sulfur occupations it was possible to identify two compatible sets of iron atoms and ligand positions. In further refinements the total population in the two molecular configurations was constrained to unity, and all phenyl groups were constrained to an idealized geometry.

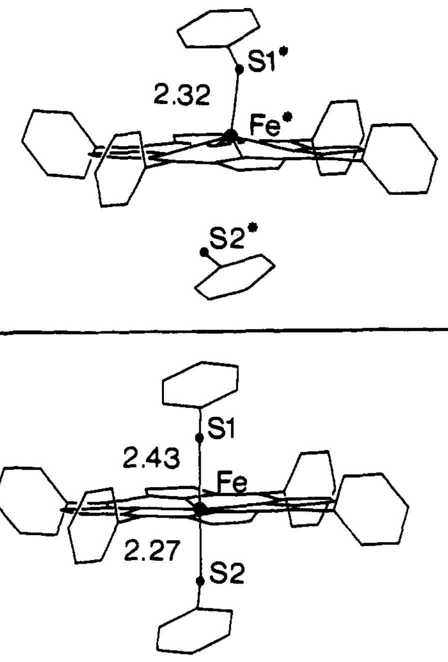

Figure 1. The high-spin and low-spin forms of Fe^{III}TPP(C_6H_5S)(C_6H_5SH).

Table I. Crystal Data

Space group $P1$; $Z = 1$
Lattice parameters (115 K)
$a = 10.678\ (3)$, $b = 11.463\ (3)$, $c = 12.305\ (5)$ Å
$\alpha = 58.37\ (2)$, $\beta = 57.04\ (2)$, $\gamma = 72.12\ (2)°$
Radiation Mo Kα, monochromatized
Crystal dimensions, 0.29 × 0.21 × 0.09 mm
Scan range, 1.0° below K$_{\alpha 1}$ to 1.0° above K$_{\alpha 2}$
Scan rate, 2.0°/min
Scan mode, $\theta/2\theta$
Background time = scan time
$2\theta_{max} = 45°$
Observed reflections ($h > 0$, $I > 3\sigma I$), 1754
$R = 0.054$, $R_w = 0.056$
Error in observation of unit weight, 1.6

The final model consists of a disordered mixture of a six-coordinate, low-spin complex[15] and a five-coordinate, high-spin complex in which the iron atom is displaced significantly from the plane of the four nitrogen atoms. The ligand lost in the transition is retained in the lattice but is not coordinated. Figure 1 shows the two structural forms identified in this investigation. At 115 K the ratio of the high-spin form to the low-spin form is 0.37/0.63. No attempt has been made to resolve alternate configurations for the porphyrin ligand, but temperature factors in the range 1.0–2.0 Å^2 indicate that the displacements of the porphyrin atoms in the course of the transition are very small.

Structural parameters of the high-spin, five-coordinate complex do not differ significantly from those obtained for iron(III) protoporphyrin IX dimethyl ester *p*-nitrophenylthiolate.[4] In particular, the Fe*-S1* distance of 2.32 (2) Å and the Fe*-S1*-C* angle of 103° [16] correspond to the values of 2.324 (2) Å and 100° in the previously reported structure.[4] On the basis of these observations as well as on electrostatic grounds, it is reasonable to infer that the uncoordinated species is benzenethiol, even though attempts to locate the thiol hydrogen in electron density difference maps have been thus far unsuccessful.

Although no other structural data are available for transition metal complexes of benzenethiol, the parameters for the low-spin complex appear to be chemically reasonable. The two Fe-S distances of 2.27 (2) and 2.43 (2) Å are in the range of distances expected for iron to benzenethiolate and iron to benzenethiol, respectively. As in the case of the high-spin complex, electron density difference maps have not produced an unambiguous identification of the thiol hydrogen atom.

The structural interpretations above provide an important insight into the mechanism of the transformation in this material. Assuming that the shorter Fe-S bond in both forms corresponds to the coordinated thi*olate*, S1 in the low-spin form appears to be protonated while S1* in the high spin form does not. This anomaly was initially attributed to a false minimum in the refinement caused by the pseudosymmetry of the complex, but numerous attempts to refine models in which the iron or axial ligand positions were interchanged resulted in convergence to the initial parameters. Thus it would appear that a proton transfer may accompany the structural transition. Speculation concerning the mechanism of such a transfer will be postponed until further studies of this complex are completed.

On the basis of the present information it is clear that the complex exists in the solid state as an equilibrium mixture of five and six-coordinate species. Crystallographic resolution of these two structural forms has produced a dynamic model for the transformation associated with the substrate binding in the catalytic cycle of P450 enzymes in which the low-spin, six-coordinate resting form is converted into a high-spin, five-coordinate species. Multiple-temperature x-ray and neutron investigations of this complex promise to provide further information related to the spin equilibria which have been observed for a number of hemoproteins and may yield, simultaneously, a detailed picture of a proton-transfer reaction.

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References and Notes

(1) The abbreviations used in this paper are as follows: P450$_{cam}$, cytochrome P450 camphor hydroxylase; TPP, tetraphenylporphyrin dianion; EPR, electron paramagnetic resonance.

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(3) For simple ferric porphyrin complexes thus far characterized, six coordination is associated with low-spin iron ($S = \frac{1}{2}$) while five coordination is indicative of a high-spin ($S = \frac{5}{2}$) configuration. For the most part this is true for the iron in hemoproteins as well; however, for very weak field ligands (F^-, H_2O), it now appears that certain six-coordinated complexes may also be high spin. See, for example, J. F. Deatherage, R. S. Loe, and K. Moffat, *J. Mol. Biol.*, **104**, 723 (1976).

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(6) Evidence has implicated a cysteine thiolate in the ferrous deoxy[7] and ferrous carbonyl[8] stages of the P450 cycle as well. A recent report disputes the possibility of thiolate ligation in the ferrous oxy form.[9] However, see ref 5b.

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(15) This complex is the *only* isolated and structurally characterized example of a six-coordinate ferric porphyrin having a thiolate ligand. Attempts to isolate such complexes by addition of a ligand to five-coordinate thiolate compounds usually result in reduction of the iron to the ferrous state, except at very low temperatures.[4,5]

(16) The Fe*-S2* distance in this form is 3.95 Å, consistent with the notion that the benzenethiol is best considered as a solvate molecule in the crystal lattice and probably exerts no influence on the electronic properties of the iron.

(17) Fellow of the Alfred P. Sloan Foundation.

James P. Collman,* Thomas N. Sorrell, Keith O. Hodgson[17]
Department of Chemistry, Stanford University
Stanford, California 94305

Ajaya K. Kulshrestha, Charles E. Strouse*
Contribution No. 3815 from the Department of Chemistry
University of California, Los Angeles
Los Angeles, California 90024

Received April 12, 1977

Pyramidal Dications. Introduction of Basal and Apical Substituents

Sir:

Recently the synthesis and the chemical behavior of $(CCH_3)_6^{2+}$, the first representative of the $(CH)_6^{2+}$ type of pyramidal dications, has been reported.[1] During our investigation on the preparation of other derivatives of this class of species we found a simple route to pyramidal dications[2] of general formula $(CCH_3)_5CR^{2+}$.

The philosophy followed to achieve this goal was to find a new precursor of $(CCH_3)_6^{2+}$ that could be synthesized by introduction of a methyl group into a starting material in which in a similar fashion other alkyl groups instead of the methyl one could easily be put. In principle the unsaturated ketone **1**,[3] easily available from the corresponding tricyclic diene, is one of the starting materials having the required characteristics.

In fact, when treated with MeLi, enone **1** provided allylic alcohol **2**,[4-6] a new precursor of dication **3**. Moreover different alkyl groups could be introduced using other Li reagents.[6]

A solution of dication $(CCH_3)_6^{2+}$ (**3**) was prepared by treating **2** with $HFSO_3/SbF_5$ (molar ratio 1:1) in SO_2ClF at −60 °C and characterized as previously reported (Scheme I).

Scheme I