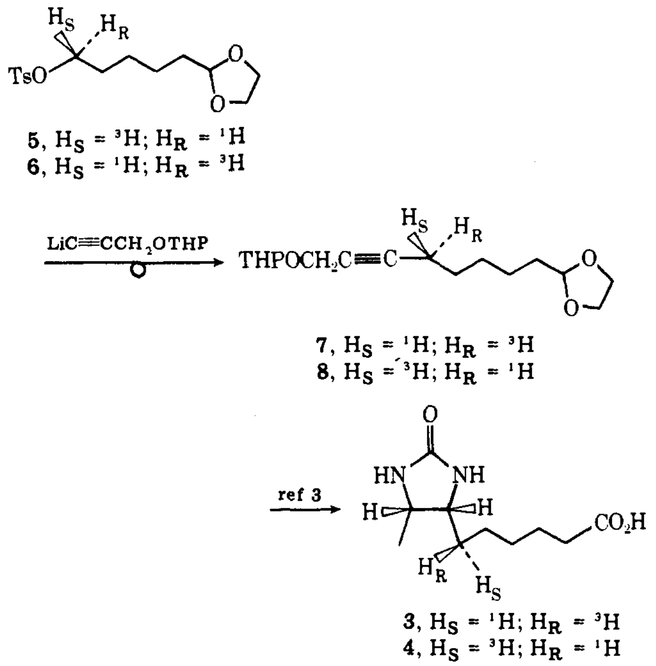
Scheme I



5, H_S = ^{3}H; H_R = ^{1}H
6, H_S = ^{1}H; H_R = ^{3}H

7, H_S = ^{1}H; H_R = ^{3}H
8, H_S = ^{3}H; H_R = ^{1}H

3, H_S = ^{1}H; H_R = ^{3}H
4, H_S = ^{3}H; H_R = ^{1}H

^{3}H]-(±)-dethiobiotin (**3** and **4**) using methods previously developed in our laboratories.[3]

The samples of chirally tritiated (±)-dethiobiotin[7] were each mixed with [10-^{14}C]-(±)-**2** and the doubly labeled dethiobiotin recrystallized to constant specific activity and constant tritium to carbon-14 ratio. The double labeled precursors were administered to cultures of *A. niger* (ATCC 1004).[8] The precursors were not resolved since only (+)-dethiobiotin appears to serve as a biotin precursor.[9] After an incubation period of 5 days, the biotin produced from each doubly labeled precursor was isolated[8] as (+)-biotin sulfone and converted into biotin sulfone methyl ester. The samples of methyl ester were recrystallized to constant specific activity and constant tritium to carbon-14 ratio. The results of the experiments are summarized in Table I.

The tritium to carbon-14 ratios of the biotin sulfone methyl ester isolated in these experiments clearly demonstrate that sulfur is introduced at C-4 of dethiobiotin with loss of the 4-*pro S* hydrogen atom. Since the absolute configuration of (+)-biotin at C-4 is known[10] to be *S*, it follows that sulfur is introduced at C-4 of dethiobiotin with *retention* of configuration at that prochiral center. This result can be compared with that of the two other examples of sulfur introduction whose stereochemistry has been elucidated. It has been established that sulfur is introduced at C-6 of octanoic acid during lipoic acid biosynthesis with *inversion* of configuration,[5] while the introduction of sulfur at C-3 of valine during penicillin biosynthesis has been found to occur with *retention* of configuration.[11] At the present time, it is not clear whether the biotin and penicillin cases proceed by a mechanism that is fundamentally different from that involved in lipoate biosynthesis, or whether the mechanism is the same in all three cases with the stereochemical outcome being dictated by other factors.[12]

Table I. Incorporation of Chirally Tritiated Dethiobiotin into Biotin

expt	precursor	^{3}H/^{14}C for precursor	^{3}H/^{14}C for biotin sulfone methyl ester	% ^{3}H retention
1	[4(*R*)-4-^{3}H-10-^{14}C]-**2**	3.37	3.07	91
2	[4(*S*)-4-^{3}H-10-^{14}C]-**2**	6.00	0.42	7

Acknowledgment. We thank the National Science Foundation (Grant CHE78-21738) for partial support of this research.

References and Notes

(1) Lynen, F. *Biochem. J.* **1967**, *102*, 381.
(2) Okumura, S.; Tsugawa, R.; Tsunada, T.; Motozaki, S. *J. Agric. Chem. Soc. Jpn.* **1962**, *36*, 599, 605. Eisenberg, M. A. *J. Bacteriol.* **1963** *86*, 673. Iwahara, S.; Kikuchi, M.; Tochikura, T.; Ogata, K. *Agric. Biol. Chem.* **1966**, *30*, 304. Eisenberg, M.; Maseda, R.; Star, C. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **1968**, *27*, 762.
(3) Parry, R. J.; Kunitani, M. G. *J. Am. Chem. Soc.* **1976**, *98*, 4024.
(4) Guillerm, G.; Frappier, F., Gaudry, M., Marquet, A. *Biochimie* **1977**, *59*, 117.
(5) Parry, R. J.; Trainor, D. A. *J. Am. Chem. Soc.* **1978**, *100*, 5243.
(6) Corey, E. J., Katzenellenbogen, J. A.; Gilman, N. W.; Roman, S. A.; Erickson, B. W. *J. Am. Chem. Soc.* **1968**, *90*, 5618.
(7) The optical purity at C-4 of **3** and **4** is ~80 and 75%, respectively. See ref 5.
(8) Li, H. C.; McCormick, D. A.; Wright, L. D. *J. Biol. Chem.* **1968**, *243*, 6442.
(9) Ogata, K. *Methods Enzymol.* **1970**, *18A*, 390.
(10) Trotter, J.; Hamilton, J. A. *Biochemistry* **1966**, *5*, 713.
(11) Neuss, N.; Nash, C. H.; Baldwin, J. E.; Lemke, P. A.; Grutzner, J. B. *J. Am. Chem. Soc.* **1973**, *95*, 3797 (corrected p 6511). Kluender, H.; Bradley, C. H.; Sih, C. J.; Fawcett, P.; Abraham, E. P. *Ibid.* **1973**, *95*, 6149. Aberhart, D. J.; Lin, L. J. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2320.
(12) For a discussion of the problems associated with the use of stereochemical information to determine the nature of enzymatic reaction mechanisms, see Retey, J. "Topics in Biochemistry of Natural Products", Swain, T., Waller, G. R., Eds.; Plenum Press: New York, 1979; pp 1–27.
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An Explanation of Unusual Properties of Spin-Crossover Ferric Complexes

Sir:

The biochemical importance of spin-state equilibria (both $S = 5/2 \rightleftharpoons S = 1/2$ and $S = 5/2 \rightleftharpoons S = 3/2$) and the $S = 3/2$ intermediate-spin state for Fe(III)-containing proteins has been noted.[1,2] A number of model ferric complexes have been reported[3,4] which exhibit the $S = 5/2$ to $S = 1/2$ spin crossover. *Solid* samples of several of these compounds exhibit unusual magnetic susceptibility characteristics. In particular, the decrease in $\mu_{\rm eff}$/Fe with decreasing temperature does not reflect a simple Boltzmann distribution over electronic states[4] and in some cases $\mu_{\rm eff}$/Fe does not decrease to a value appropriate for a low-spin ($S = 1/2$) state at low temperature.[3,5] In these latter complexes, the spin-crossover phase transition appears to be incomplete and below a certain temperature $\mu_{\rm eff}$/Fe remains relatively constant at a value intermediate between high spin and low spin. A "plateau" in the $\mu_{\rm eff}$/Fe vs. temperature curve is seen. In the case of the dichloromethane solvate of tris(4-morpholinecarbodithioato-*S*,*S*′)iron(III), for example, the $\mu_{\rm eff}$/Fe vs. T curve plateaus at a value of 3.7–3.9 μ_B below ~60 K. An intermediate-spin ($S = 3/2$) $^4T_1$ ground state was proposed[8] for this FeS_6 complex.

Interesting observations have been made in our laboratories recently that shed light on the nature of the incomplete (i.e., plateau in $\mu_{\rm eff}$/Fe) spin-crossover phase transitions observed for solid samples of ferric complexes. A series of complexes with the composition $[Fe^{III}(X\text{-}SalEen)_2]Y$, where X-SalEen is the monoanion of the condensation product of salicylaldehyde (or a methoxy-substituted salicylaldehyde) and *N*-ethylethylenediamine, and Y is variously NO_3^-, PF_6^-, or BPh_4^-, was prepared. These compounds were precipitated as microcrystalline solids from methanol. No evidence of solvation

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by methanol was noted. We encountered in this series of compounds the three main types of μ_{eff}/Fe vs. temperature curves. The compound [Fe(SalEen)$_2$]PF$_6$ undergoes a gradual, but complete, spin-crossover transition, whereas the NO$_3^-$ salt of the same cation exhibits a "plateau" at 3.03 μ_B in the μ_{eff}/Fe vs. temperature curve below 77 K. The plateau for [Fe(3-OCH$_3$-SalEen)$_2$]BPh$_4$ is even more pronounced in the sense that μ_{eff}/Fe for this compound varies from 5.92 μ_B at 286 K to 4.95 μ_B at 4.2 K. A sudden transition from high spin to low spin within a few degrees is seen for [Fe(3-OCH$_3$-SalEen)$_2$]-PF$_6$.

Three important observations were made on the [Fe(X-SalEen)$_2$]Y compounds. First, and perhaps most interesting, is the discovery that grinding one of the microcrystalline [Fe(X-SalEen)$_2$]Y compounds generally yields a compound with a "plateauing" μ_{eff}/Fe vs. temperature curve. Figure 1 shows the results for [Fe(3-OCH$_3$-SalEen)$_2$]PF$_6$. A particular microcrystalline sample of this compound was divided into three parts. Variable-temperature magnetic susceptibility data collected for one unperturbed fraction show a sharp, *complete* phase transition. A second sample was ground with a mortar and pestle and then data were collected. The third fraction was ground even more thoroughly with a ball mill. It can be seen in Figure 1 that both of the ground samples exhibit plateauing μ_{eff}/Fe vs. temperature curves. The most thoroughly ground sample plateaus at the higher value of μ_{eff}/Fe = ~2.80 μ_B. The spin-crossover phase transition observed is not complete and a certain percentage of the molecules in this sample persists in the high-spin state. It is important to note that we find the effects of grinding a sample to be reversible. Dissolving the ground sample in methanol and evaporating the solution to dryness gives microcrystals which behave the same as the original unground material.

The second type of observation that we made bears on the question of whether certain of these complexes have a $^4T_1$ ground state. Considerable variable-temperature (room temperature to 4.2 K) ^{57}Fe Mössbauer and EPR data have been collected which, together with the susceptibility data, show that none of our spin-crossover ferric complexes have a $^4T_1$ ground state. Each complex which has a plateauing μ_{eff}/Fe vs. temperature curve gives a Mössbauer (EPR) spectrum in the plateauing region with two signals, one for the molecules that are low-spin ($S = 1/2$) and one for the molecules that persist in the high-spin ($S = 5/2$) state. The relative areas of the two signals in a given spectrum parallel the bulk magnetic susceptibility as the temperature of the compound is varied.

Subtle is the best description of the third type of observation made on the [Fe(X-SalEen)$_2$]Y complexes. An examination of Figure 1 shows that, as the degree of grinding (plateauing) increases from one sample to another, the phase transition becomes more gradual. That is, the slope of the μ_{eff}/Fe vs. temperature curve decreases with increased grinding. A similar observation is made for the [Fe(X-SalEen)$_2$]Y compounds doped into isostructural Co(III) and Cr(III) compounds. We find that, as the percentage of [Fe(X-SalEen)$_2$]Y is decreased in a series of samples doped into either a cobalt or chromium host, the phase transition is observed to become more gradual.

It is important to point out that some of the same types of observations have been reported for spin-crossover Fe(II) complexes where the transition is from an $S = 2$ state to an $S = 0$ state. The effect upon the phase transition of grinding a sample has *not* been noted previously. Plateauing has been noted for Fe(II) complexes and in some cases it has been attributed to a permanent paramagnetic impurity. Sorai and Seki[6] studied Fe^{II}(phen)$_2$X$_2$, where X is either NCS$^-$ or NCSe$^-$, and attributed the plateau in μ_{eff}/Fe vs. temperature as "high-spin residue" due to molecules that resist conversion into low spin. Doping spin-crossover Fe(II) complexes into zinc hosts has been seen to make the transition more gradual.[7] Very recently, Gütlich et al.[8] described a phenomenological thermodynamic model that takes into account domain formation. In this model domain size and cooperativity enthalpy are the most important factors in determining the nature of the spin-crossover transition.

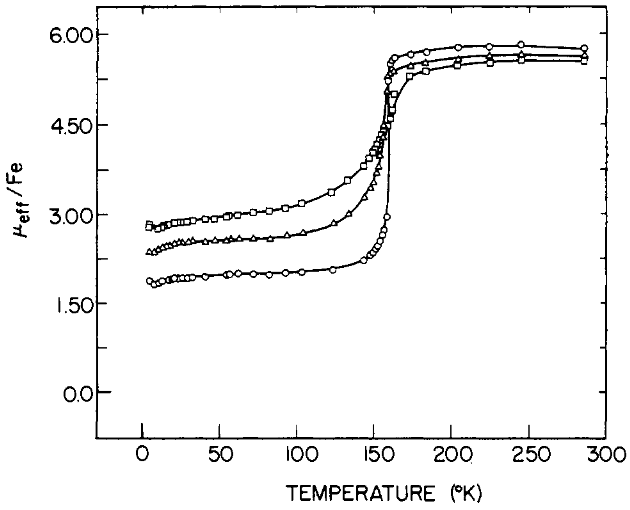

Figure 1. Effective magnetic moment per iron ion (μ_{eff}/Fe) vs. temperature curves for [Fe(3-OCH$_3$-SalEen)$_2$]PF$_6$: ○, original microcrystalline solid; △, same solid ground with motar and pestle; □, same solid ground in a ball mill.

It appears that all of the effects of grinding or doping a spin-crossover complex and the factors that determine whether a given complex exhibits a gradual or sudden transition (i.e., non-Boltzmann behavior) can be explained by the nucleation and growth mechanism of phase transitions in solids.[9,10] There is kinetic control on the spin-crossover phase transition. The transformation from high spin to low spin involves the formation of nuclei (small regions of molecules) of the low-spin phase in the high-spin phase and the subsequent growth of the nuclei. Before a nucleus can persist and continue to grow, it has to attain a critical size. The rate of growth of critical-sized nuclei of low-spin molecules determines the nature of the spin-crossover phase transition. Defects, grain boundaries, vacancies, and other imperfections in the solid are preferred sites for nucleation. This mechanism requires the coexistence of both phases around the transition temperature. Mössbauer and EPR data for the [Fe(X-SalEen)$_2$]Y complexes (even the sudden system) do show signals for both phases in the transition temperature region.

Grinding a [Fe(X-SalEen)$_2$]Y complex probably leads to an increase in defects and stress points in the crystals. This leads to an increase in nucleation sites and a lowering of the μ_{eff}/Fe value at higher temperatures as the degree of grinding is increased (see Figure 1). It is known that the critical size of a nucleus decreases with decreasing temperature.[12,13] The plateau in the μ_{eff}/Fe vs. temperature curve at an intermediate value is due to an increase in the activation barrier for the transformation. The activation barrier is increased in the ground sample because there is impingement between growing nuclei which are more abundant in the ground sample than in the unground sample. A larger activation energy could also be present in the ground sample because growing nuclei will more frequently encounter defects that will deter the growth of the minority-spin nucleus.

The effects of doping a [Fe(X-SalEen)$_2$]Y complex into either a Cr(III) or a Co(III) host can also be understood by the same nucleation mechanism. As the percentage of host molecules is increased, the phase transition becomes more gradual. When a growing nucleus of low-spin Fe(III) complexes encounters a host molecule, this increases the activation energy

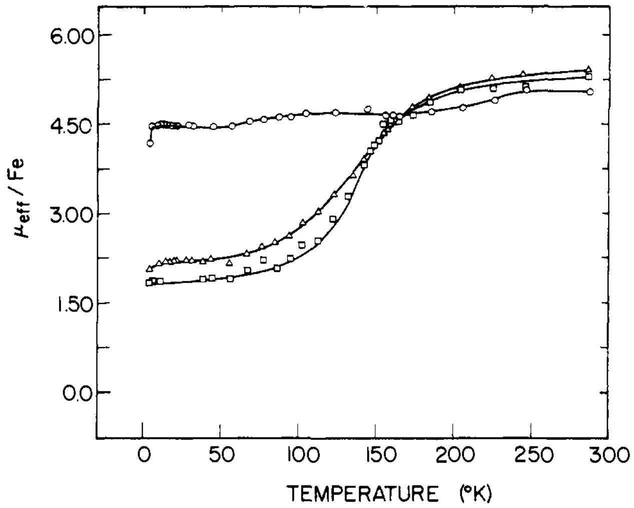

Figure 2. Effective magnetic moment per iron ion (μ_{eff}/Fe) vs. temperature curves for $[Fe_{0.5}Cr_{0.5}(3\text{-}OCH_3\text{-}SalEen)_2]PF_6$: △, original microcrystalline solid; ○, microcrystalline solid ground in ball mill; □, ground sample recrystallized by evaporating a methanol solution to dryness.

for the transformation. We have found that the most pronounced effect of grinding is observed for a doped sample. Figure 2 shows the results for $[Fe_{0.5}Cr_{0.5}(3\text{-}OCH_3\text{-}SalEen)_2]PF_6$. The original microcrystalline sample shows a gradual but complete transition. When the same sample is ground in a ball mill, a large fraction of the sample does *not* undergo the transition and a plateau develops. Recrystallization of this ground sample from methanol gives a sample that behaves as the original sample.

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References and Notes

(1) (a) Iizuka, T.; Yonetani, T. *Adv. Biophys.* **1970**, *1*, 157. (b) Dose, E. V.; Tweedle, M. F.; Wilson, L. J.; Sutin, N. *J. Am. Chem. Soc.* **1977**, *99*, 3887, and references therein.
(2) Maltempo, M. M.; Moss, T. H. *Q. Rev. Biophys.* **1976**, *9*, 181, and references therein.
(3) Petty, R. H.; Dose, E. V.; Tweedle, M. F.; Wilson, L. J. *Inorg. Chem.* **1978**, *17*, 1064.
(4) Hall, G. R.; Hendrickson, D. N. *Inorg. Chem.* **1976**, *15*, 607.
(5) Butcher, R. J.; Sinn, E. *J. Am. Chem. Soc.* **1976**, *98*, 5159.
(6) Sorai, M.; Seki, S. *J. Phys. Chem. Solids* **1974**, *35*, 555.
(7) Gütlich, P.; Link, R.; Steinhauser, H. G. *Inorg. Chem.* **1978**, *17*, 2509. Sorai, M.; Ensling, J.; Gütlich, P. *Chem. Phys.* **1976**, *18*, 199.
(8) Gütlich, P.; Köppen, H.; Link, R.; Steinhäuser, H. G. *J. Chem. Phys.* **1979**, *70*, 3977.
(9) Rao, C. N. R.; Rao, K. J. "Phase Transitions in Solids"; McGraw-Hill: New York, 1978.
(10) Christian, J. W. "The Theory of Transformations in Metals and Alloys", 2nd ed.; Pergamon Press: Oxford, 1975; Part I.
(11) University of Illinois Fellow, 1976–1979.
(12) 3M Fellowship, 1979–1980.
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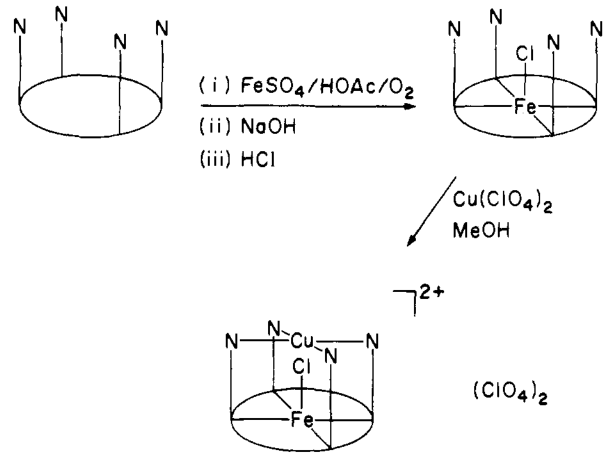
Toward Synthetic Models for Cytochrome Oxidase: a Binuclear Iron(III) Porphyrin-Copper(II) Complex

Sir:

The structure and function of the active site of cytochrome *c* oxidase has been the subject of much investigation and controversy over the past 40 years.[1] More recent ESR,[2] magnetic susceptibility,[3] and mcd[2,4] measurements have indicated the presence of a strongly magnetically coupled Fe(III)–Cu(II) center at the cytochrome a_3 active site in the fully oxidized enzyme. Because of the problems involved in studying the natural enzyme, and the paucity of available information on simple binuclear systems containing different metal ions, there has been considerable recent interest in the synthetic analogue approach to the active site structure of cytochrome *c* oxidase.[5-9]

Scheme I



(1)

We have reported[8] the synthesis and some preliminary studies on a binuclear model consisting of a porphyrin with a covalently attached tetrapyridine ligand system, *meso*-$\alpha,\alpha,\alpha,\alpha$-tetra(*o*-nicotinamidophenyl)porphyrin, (P)–(N_4). We now report synthetic, structural, and magnetic data for the Fe(III)–Cu(II) mixed metal system, $[Fe(P)\text{-}Cl\text{-}Cu(N_4)](ClO_4)_2$.

Insertion of Fe into the porphyrin to give $Fe(P)Cl\text{-}(N_4)$[10] was achieved by the ferrous sulfate–acetic acid method,[11] followed by treatment of the crude reaction mixture sequentially with aqueous NaOH and 0.01 N HCl. Treatment of a chloroform–methanol (9:1) solution of this compound with a methanolic solution of cupric perchlorate (1 mol) and recrystallization from aqueous acetonitrile gave the dihydrate $[Fe(P)\text{-}Cl\text{-}Cu(N_4)](ClO_4)_2\cdot 2H_2O$[12] (**1**) (Scheme I).

After many unsuccessful attempts, single crystals of **1** were isolated as small, poorly formed brown blocks, most of which were twinned, and none of which diffracted well. Despite the poor quality of the crystals, we were able to obtain X-ray diffraction data as follows: space group $P4/n$; $Z = 2$; cell dimension $a = 15.96$ (1), $c = 14.36$ (1) Å. Intensity data were collected on a Picker FACS1 4-circle diffractometer using graphite-monochromated Mo Kα radiation and θ–2θ scan technique. Of the 1980 unique reflections collected up to 2θ maximum (Mo Kα) of 40°, only 720 (36%) with $I > 2\sigma(I)$ were considered to be observed. The structure was solved by direct methods and refined by full-matrix least squares. The anions were not clearly resolved and are disordered around the fourfold axis. A second water molecule was also unresolved. The current residuals R and R' are 0.214 and 0.234 for the limited number of data and 93 variables.[13,14]

A perspective view of the cation is shown in Figure 1. It has fourfold symmetry with the Fe, Cu, and Cl atoms lying on the fourfold axis. The Cu is coordinated to the four pyridine nitrogens, and the chloride ion lies midway between the Fe and Cu atoms. The exceptionally long Cu–Cl and Fe–Cl bonds (average 2.47 (5) Å) indicate weak bridging between the two metals. The Fe atom is located essentially in the mean plane of the porphyrin and is octahedrally coordinated with a water molecule occupying the sixth coordination site (Fe–O = 2.09

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