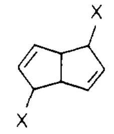
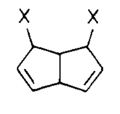
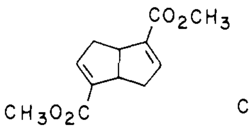


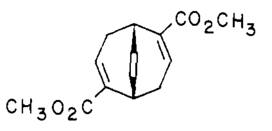
8



9



10



11

aqueous potassium carbonate at ambient temperature. Perhaps this reflects the greater anionic stabilization to be expected in the longicyclic environment.

A sharper distinction results from the addition of 12-crown-4[19] to tetrahydrofuran solutions of the ionic [3.3.2] and the covalent [3.3.0] organolithium compounds at −78 °C. Subsequent deuteronation of the [3.3.2] solution introduced 1.97 (2) atoms of deuterium, indistinguishable from the 1.95 (4) atoms acquired in the absence of crown ether.[1] Subsequent deuteronation of the [3.3.0] solution, however, introduced only 0.80 (5) atoms of deuterium, half as much as in the absence of crown ether. We assume that this difference reflects, first, the ability of the crown ether to displace the destabilized bicyclo[3.3.0]octadienyl ligand (**5**) from the coordination sphere of lithium, and, second, the unusual basicity of the free ligand. It apparently deprotonates the solvent and/or crown ethers to provide the allylically stabilized bicyclo[3.3.0]octenyl anion. Only one deuteron can then be acquired.

If that be so, **5** cannot be an intermediate in the thermal rearrangement of dilithium semibullvalenide to the cyclooctatetraenyl dianion, a reaction that proceeds with $10^5 k^{0\,°C} = 9.0\ (1)$[20] and without detectable byproducts. As expected, the stabilized bicyclo[3.3.2]decatrienyl dianion (**2**) is quite inert under these same conditions.

These results suggest that the binary predictions of qualitative models[2,3]—stabilized or destabilized—might more generally be reflected in the binary properties of organolithium compounds—ionic or covalent. Previous failures to detect anionic manifestations of the homoaromatic model[4a–d,h] have many possible origins. Perhaps these new results will encourage a more critical reexamination of the older data and their interpretations. The failures of ab initio STO-4G calculations to detect "through-space" overlap, in homoaromatic and longicyclic anions,[4k,l] are easier to understand. Such minimal basis-set models are notoriously unreliable guides to the thermodynamic properties of gas-phase carbanions.[21,22] We are encouraged that one group has since turned to quantitatively more realistic models,[23] and we wish them well.

Acknowledgment. We are grateful to N. J. Turro and H. E. Zimmerman for a preprint of ref 6 in advance of publication and to the National Science Foundation for its support of the research (CHE77-26482) and of NMR facilities (Varian CFT-20, CHE76-05884, and Bruker WM-300, CHE79-04825, at Cornell, and Bruker HX-270, CHE79-16210, at the Southern New England NMR Facility).

Registry No. 1, 1005-51-2; **2**, 81408-59-5; **4**, 6909-37-1; **5**, 81408-60-8; **8** (x = H), 17618-97-2; **8** (X = CO_2H), 81408-61-9; **9** (X = H), 7863-35-8; **10**, 81408-62-0; **11**, 81408-63-1.

---

[18] **11**: mp 100.5 °C; IR (CCl_4) 1715 cm^{-1}; $\delta_H^{CDCl_3}$ 7.08 (1.9 H, t, J = 4 Hz), 6.07 (1.9 H, dd, J = 4, 6 Hz), 3.7 (8.1 H, s within m), 2.58 (4.1 H, m).

[19] Anet, F. A. L.; Krane, J.; Dale, J.; Daasvatn, K.; Kristiansen, P. O. *Acta Chem. Scand.* **1973**, *27*, 3395–3402.

[20] An apparent first-order rate constant. More thorough analyses are in progress.

[21] Cãrsky, P.; Urban, M. "Ab Initio Calculations"; Springer: Berlin, 1980; pp 74–76, 151–154. Schaefer, H. F., III. *Ann. Rev. Phys. Chem.* **1976**, *27*, 261–290. Radom, L. In "Modern Theoretical Chemistry"; Schaefer, H. F., III, Ed.; Plenum Press: New York, 1977.

[22] Pross, A.; DeFrees, D. J.; Levi, B. A.; Pollack, S. K.; Radom, L.; Hehre, W. J. *J. Org. Chem.* **1981**, *46*, 1693–1699.

[23] Clark, T.; Schleyer, P. v. R.; Houk, K. N.; Rondan, N. G. *J. Chem. Soc., Chem. Commun.* **1981**, 579–581. Schleyer, P. v. R.; Chandresekhar, J. C.; Kos, A. J.; Clark, T.; Spitznagel, G. W. Ibid. **1981**, 882–884. Chandresekhar, J.; Andrade, J. G.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1981**, *103*, 5609–5612.

A Trimeric Iron(III) Heme-Copper(II) Complex: Support for an Alternate Explanation of the "EPR Silent" Iron-Copper Pair in Cytochrome *c* Oxidase

C. Michael Elliott* and Kozo Akabori

Department of Chemistry, Colorado State University
Fort Collins, Colorado 80523

Received November 30, 1981

One of the major enigmas associated with the active site of cytochrome *c* oxidase is the so-called "missing" or "EPR silent" ferric heme-copper pair in the resting enzyme. Recently, the prevalent view has been that the absence of an observable EPR signal from both the iron and copper is best explained by proposing a high-spin ($S = {}^5/_2$) ferric heme strongly antiferromagnetically coupled to the ($S = {}^1/_2$) copper.[1,2] This model is consistent not only with EPR results but with the bulk magnetic susceptibility, which is significantly lower than what would be expected for the same system with isolated spins.[3–6]

The major problem with this model is that in order for it to explain the lack of an observed temperature dependence in the magnetic moment, an exceptionally large coupling constant, $-J > 200\ cm^{-1}$, must exist.[1,2,4–6] The problem is further complicated by the recent proposal of a detailed active-site structure based on EXAFS data for the enzyme.[7] In that study the coupled Fe/Cu pair is described as being bridged by a cysteine-like thiolate. If this structural representation is correct, the uncomfortable fact remains that a sulfur-mediated coupling of the order of magnitude of $-200\ cm^{-1}$ is completely unprecedented.[8] The preparation of structurally appropriate iron–copper-containing model complexes is important to further the understanding of the physical and chemical properties of the enzyme, especially those intimately related to spin states.

Using simple monomeric metal complexes, we have taken advantage of established properties of iron hemes and prepared a unique complex that incorporates two irons (porphyrins) and one copper into a single trimeric moiety. Treatment of *meso*-tetraphenylporphyrinatoiron(II) ($Fe^{II}TPP$) with a stoichiometric equivalent of tetrabutylammonium bis(*cis*-1,2-dicyano-1,2-ethylenedithiolato)cuprate(III) ($TBA^+Cu^{III}MNT_2^-$) in dry benzene under N_2 results in a reaction having the following stoichiometry:

$$Fe^{II}TPP + TBA^+Cu^{III}MNT_2^- \rightarrow {}^1/_2(TBA^+)_2Cu^{II}MNT_2^{2-} + {}^1/_2Fe^{III}TPP\cdot[Cu^{II}MNT_2]\cdot Fe^{III}TPP$$

1

Compound **1** is isolated as black crystals or as a purple-black powder. (Anal. Calcd for $C_{96}H_{56}N_{12}S_4Fe_2Cu$: C, 68.59; H, 3.36; N, 10.00. Found: C, 68.52; H, 3.33; N, 9.78). Molecular weights

---

[1] Palmer, G.; Babcock, G. T.; Vickery, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2206.

[2] Reed, C. A.; Landrum, J. T. *FEBS Lett.* **1979**, *106*, 265.

[3] Van Gelder, B. F.; Beinert, H. *Biochim. Biophys. Acta* **1969**, *189*, 1.

[4] Tsudzuki, T.; Okunuki, K. *J. Biochem.* **1971**, *69*, 909.

[5] Tweedle, M. F.; Wilson, L. J,; Garcia-Iniguez, I.; Babcock, G. T.; Palmer, G. *J. Biol. Chem.* **1978**, *253*, 8065.

[6] Mass, T. H.; Shapiro, E.; King, F. E.; Beinert, H.; Hartzell, C., *J. Biol. Chem.* **1978**, *253*, 8072.

[7] Powers, L.; Chance, B.; Ching, Y.; Angiolillo, P. *Biophys. J.* **1981**, *34*, 465.

[8] Iron sulfur clusters have been shown to exhibit $-J > 200\ cm^{-1}$ (see Holm, R. et al. *J. Am. Chem. Soc.* **1978**, *100*, 5322) but in these cases the Fe–Fe distances are so short (2.7 Å) that direct exchange is likely.

0002-7863/82/1504-2671$01.25/0

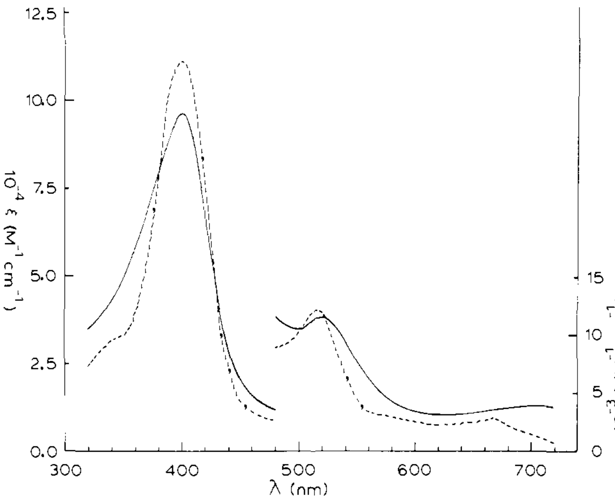


Figure 1. Comparison of visible spectrum of $[Fe^{III}TPP]_2 \cdot Cu^{II}MNT_2$ (—) with that of $Fe^{III}TPP \cdot OClO_3$ (---), both in CH_2Cl_2 solution. The spectrum of $[Fe^{III}TPP]_2 \cdot Cu^{II}MNT_2$ is plotted for the molar extinction coefficient per porphyrin unit.

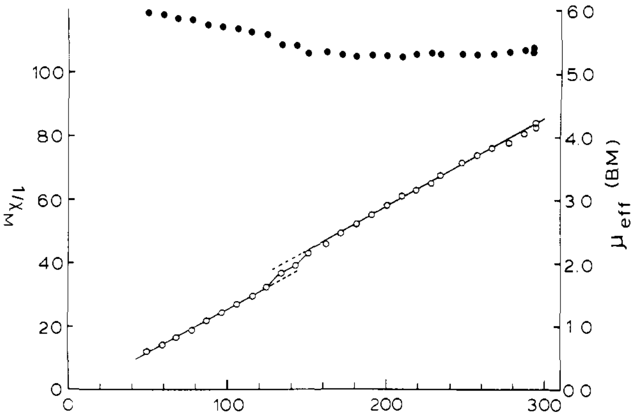


Figure 2. Inverse molar magnetic susceptibility in cgsu (●) and effective magnetic moment (per trinuclear complex) in μ_B (○) vs. temperature curve for $[Fe^{III}TPP]_2 \cdot Cu^{II}MNT_2$.

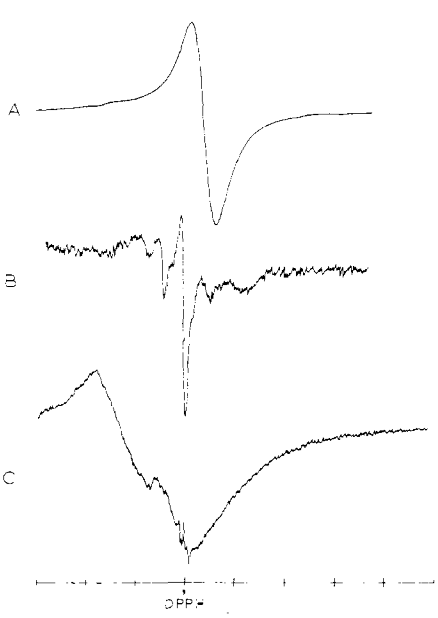


Figure 3. Solid state EPR spectra of (A) $(TBA^+)_2Cu^{II}MNT_2^{2-}$ at room temperature, x axis = 100 G/division, relative effective gain = X1, (B) $[Fe^{III}TPP]_2 \cdot Cu^{II}MNT_2$ at 85 K, x axis = 100 G/division, relative effective gain = X226, (C) $[Fe^{III}TPP]_2 \cdot Cu^{II}MNT_2$ at room temperature, x axis = 1000 G/division, relative effective gain = X3150. Each spectrum has been "centered" on the resonance of a DPPH external standard as indicated.

calculated from atomic absorption analysis of Fe and Cu substantiate a trinuclear formulation for the product (calculated mol wt = 1681.08; found, 1680 ± 60 from Fe_2, 1650 ± 50 from Cu). In noncoordinating solvents (e.g., CH_2Cl_2, C_6H_6, $C_6H_5NO_2$) **1** is only very slightly soluble ($\sim 10^{-5}$ M). In solution the complex is rapidly oxidized by O_2, but in the solid it is stable (no oxidation is observed from visible spectra after exposure to air after a 5-month period).

In coordinating solvents (e.g., THF, DMF, Me_2SO) **1** is quite soluble and, based on differences in the visible spectrum, probably exists in a partially dissociated form.

In each of the noncoordinating solvents investigated, the visible spectra of **1** (Figure 1) are quite similar. In general the spectrum of **1** resembles those of various five-coordinate iron porphyrins having a significant amount of intermediate spin[9] ($S = {}^3/_2$) character[10,11] more closely than either high-spin[12-16] or low-spin[17-19] five-coordinate iron hemes. Distinct differences also exist between the spectrum of **1** and reported spectra of six-coordinate low[20,21] and high-spin[22,23] ferric porphyrins.

The magnetic susceptibility of complex **1** was determined over the temperature range 300–50 K with a Faraday balance. Plots of χ_M^{-1} and μ_{eff} (per trinuclear unit) vs. T (K) are shown in Figure 2. The measured moment remains almost constant at 5.31 ± 0.03 μ_B in the region 300–150 K. In the range of 150–120 K the moment undergoes a small increase (+0.3 μ_B), which we have interpreted as being a simple crystal-lattice change although this has not yet been confirmed. Below 120 K the moment increases gradually from 5.6 μ_B at 120 K to 5.9 μ_B at 50 K. The χ_M^{-1} vs. T plots in the ranges 300–150 and 120–50 K each show Curie–Weiss behaviors with small Θ values (the former, 3.7 K; the latter, −3.7 K). The evidence seems to rule out the possibility of an antiferromagnetically coupled system having a thermally accessible high-spin state as is found, for example, in $(TPPFe)_2O$.[24]

The calculated spin-only moment for the two Fe(III) ($S = {}^3/_2$) and one Cu(II) ($S = {}^1/_2$) for a completely noncoupled system is 5.74 μ_B. The spin-only values for a similar low-spin and high-spin iron system are 3.00 and 8.54 μ_B, respectively. The magnitude of the spin-only moment and the lack of a thermally accessible spin-state change are consistent with the visible spectra and again strongly suggests that two intermediate-spin ($S = {}^3/_2$) iron(III) species and a ($S = {}^1/_2$) copper(II) are present in the complex.[25]

---

[9] In the majority of iron porphyrin cases where the $S = {}^3/_2$ state is important, the spin state is actually a quantum mechanical admixture of $S = {}^3/_2$ and $S = {}^5/_2$ (e.g., $Fe^{III}(TPP)ClO_4$, $S = 3/2$ with approximately 35% $S = 5/2$ admixture). The trimeric complex reported here, based on susceptibility data, appears to be most predominantly $S = 3/2$.

(10) Reed, C. A.; Mashiko, T.; Bentley, S. P.; Kastner, M. E.; Scheidt, W. R.; Spartalian, K.; Lang, G. *J. Am. Chem. Soc.* **1979**, *101*, 2948.

(11) Baldwin, J. E.; Haraldsson, G. G.; Jones, J. G. *Inorg. Chim. Acta* **1981**, *51*, 29.

(12) Hoard, J. L.; Cohen, G. H.; Glick, M. D. *J. Am. Chem. Soc.* **1967**, *89*, 1992.

(13) Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1977**, *30*, 2655.

(14) Hatano, K.; Scheidt, W. R., *Inorg. Chem.* **1979**, *18*, 877.

(15) Anzai, K.; Hatano, K.; Lee, Y. J.; Scheidt, W. R. *Inorg. Chem.* **1981**, *20*, 2337.

(16) Kessel, S. L.; Hendrickson, D. N. *Inorg. Chem.* **1980**, *19*, 1883.

(17) Summerville, D. A.; Cohen, I. A. *J. Am. Chem. Soc.* **1976**, *98*, 1747.

(18) Scheidt, W. R.; Summerville, D. A.; Cohen, I. A. *J. Am. Chem. Soc.* **1976**, *98*, 6623.

(19) Kadish, K. M.; Cheng, J. S.; Cohen, I. A.; Summerville, D. A. In "Electrochemical Studies of Biological Systems"; Sawyer, D. T. Ed.; ACS Symposium Series No. 38, American Chemical Society: Washington, D.C., **1977**; Chapter 5.

(20) Mashiko, T.; Reed, C. A.; Haller, K. J.; Kastner, M. E.; Scheidt, W. R. *J. Am. Chem. Soc.* **1981**, *103*, 5758.

(21) Byrn, M. P.; Strouse, C. E. *J. Am. Chem. Soc.* **1981**, *103*, 2633.

(22) Walker, F. A.; Lo, M.; Ree, M, T. *J. Am. Chem. Soc.* **1976**, *98*, 5552.

(23) Mashiko, T.; Kastner, M. E.; Spartalian, K.; Scheidt, W. R.; Reed, C. A. *J. Am. Chem. Soc.* **1978**, *100*, 6354.

(24) Boyd, P. D. W.; Smith, T. D. *Inorg. Chem.* **1971**, *10*, 2041.

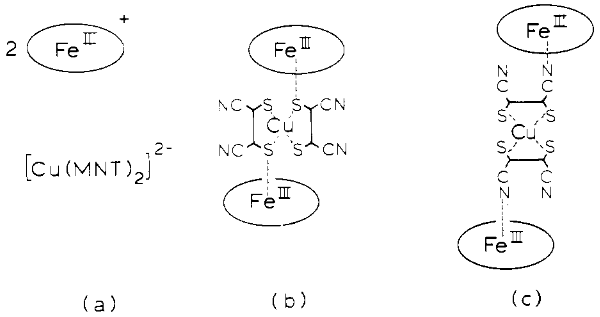


Figure 4. Possible structures of $[Fe^{III}TPP]_2 \cdot Cu^{II}MNT_2$.

The solid state EPR of **1** shows only a very weak signal, both at room temperature and 85 K. The spectra have basically two features. First, there is a variable-intensity (from sample to sample) sharp signal centered at about $g = 2$ (Figure 3B), which is almost identical in appearance with the frozen methanol glass spectrum of $Cu^{II}MNT_2^{2-}$.[26] Typically, the intensity of the signal corresponds to between 10^{-3} and 10^{-4} times that of an equivalent molar quantity of authentic $(TBA^+)_2Cu^{II}MNT_2^{2-}$ (cf. Figure 3A,B). Based on the shape, variability of intensity, and weakness of this signal, it seems most likely that it arises from a small amount (<0.1%) of a microcrystalline $(TBA^+)_2Cu^{II}MNT_2^{2-}$ impurity occluded in the crystal masses of **1** and not from an intrinsic signal of **1**. The second feature of the spectrum is an exceedingly broad (about 2000 G wide at room temperature) signal with an approximate g value of 2.6 (Figure 3C). The maximum amplitude of the derivative signal for this transition is several orders of magnitude smaller than that of the pure $(TBA^+)_2Cu^{II}MNT_2^{2-}$ sample. Cooling the sample to 85 K causes no drastic changes in the EPR spectrum, only slight increases in intensity of both signals, and an increased broadening of the $g = 2.6$ signal. Because of the low solubility of **1** in noncoordinating solvents, frozen solution spectra are difficult to obtain. Nonetheless, dilute frozen benzene solutions (77 K) of **1** produce the same results, within experimental error, as the solid. The very low-intensity EPR signal for either the iron or the copper cannot be based on a large antiferromagnetic coupling. Proposing such a coupling would be inconsistent with the visible spectra, Mössbauer spectra (vide infra), and most importantly the bulk susceptibility. It instead appears that the near "EPR silence" of **1** can best be explained by invoking a combination of dipolar coupling and a small exchange coupling between the copper and the rapidly relaxing $S = {}^3/_2$ irons.[27] This combination of couplings provides a mechanism whereby the copper spin can be relaxed and the EPR signal broadened almost beyond the point where it is experimentally observable.

The preliminary Mössbauer spectra[28] of **1** at both room and liquid helium temperature show the presence of a single type of iron. At room temperature the spectra consists of a sharp, well-resolved quadrupole doublet with a large quadrupole splitting (ΔE_Q (mm/s) = 3.13; δ (mm/s) = 0.24 relative to Fe^0). At 4.2 K a well-resolved paramagnetic hyperfine structure is observed in a small applied field. The splitting and isomer shift are consistent with the assigned $S = {}^3/_2$ spin for both irons.

The solid-state infrared spectrum of **1** shows a single sharp CN stretch at 2200 cm^{-1}, corresponding closely to that of $(TBA)^+_2Cu^{II}MNT_2$ (2198 cm^{-1}).

Based on the known stoichiometry for **1** the most reasonable structural representations are shown in Figure 4. With respect to structure **4a**, to date no structurally characterized four-coordinate iron(III) porphyrins have been reported. This fact in conjunction with the various observations above have caused us to dismiss this representation as unlikely.

No single piece of data presently available is sufficient to allow either the "S"- or the "CN"-bridged structures to be totally eliminated from consideration. In support of the CN-bridged structure are the facts that (1) structure **4c** would provide the most sterically favorable mode of binding and (2) at least one example of an intermediate-spin iron porphyrin with an N-bound nitrile-containing anion has been reported.[29] On the other hand, if the binding is via the nitrogen and if that binding is moderately strong, one would expect to see a significant shift in the stretching frequency in the IR for the bridging CN's. This is clearly not observed.

A piece of evidence in support of sulfur bridging in **1** (Figure 4b) comes from preliminary EXAFS studies.[30] A single "first shell" neighbor of the irons located at 2.44 Å has been identified tentatively as a sulfur. Additionally, a structure such as **4b** is not totally without precedent. Reaction between $Co^{II}TPP$ and bis(1,2-bis(trifluoromethyl)-1,2-ethylenedithioato)nickelate(III) produces an analogous Co–S–Ni bridged dimer that has been structurally characterized.[31,32] When all of the data are considered together, we feel that the stronger case can be made in support of the sulfur-bridged structure. An unambiguous structural assignment, however, will have to await the X-ray crystal structure determination, which is in progress.

Several examples of ferric heme–copper complexes have now been reported.[33-35] These have included examples of both $S = {}^1/_2$ and $S = {}^5/_2$ iron, but in no case has any strong exchange coupling been observed, certainly nothing of the order of −200 cm^{-1}. Other examples, however, of extensive relaxation broadening have been observed,[33-35] and in each of these cases there is evidence of some amount of $S = {}^3/_2$ spin associated with the iron. It would appear then that iron relaxation of a copper spin is not unique to **1**. For these other model systems, EPR spectra are observable but only at sufficiently low temperatures. While EPR measurements below 77 K have not yet been attempted for **1**, Mössbauer spectra (vide supra) indicate that a slow iron relaxation is occurring also for **1** at 4.2 K. There are examples, however, of simple $S = {}^3/_2$ ferric hemes[9] for which relaxation rates are reported to be exceedingly fast at 4.2 K even in the presence of a large applied magnetic field.[36] The existence of such rapidly relaxing simple systems together with the observed ability of iron to effectively relax the copper spin within the model complexes raises a question about the origin of the "EPR silence" in the resting oxidase. Carter et al.,[37] based on resonance Raman and

---

(25) All of the $S = {}^3/_2$ iron porphyrin systems that have been reported thus far have susceptibilities that significantly exceed the spin-only value. This may in part be due to $S = {}^5/_2$ admixture. Even in the case of the supposed pure $S = {}^3/_2$ systems, $Fe^{III}TPP \cdot C(CN)_3$, the measured susceptibilities are much greater than the spin-only value, thus indicating a large orbital contribution. We can only speculate at this point why $[FeTPP]_2CuMNT_2$ apparently lacks this orbital contribution. The bulkiness of the axial ligand, its relatively low symmetry at the possible binding sites (S or CN) or its potential to back bond are all possible factors that could effect the relative energies of the iron d orbitals in a way that could ultimately reduce their contributions to the susceptibility. Finally, while other iron(III) porphyrins have μ values exceeding the spin-only value, there are several examples with nonporphyrin five-coordinate iron where $S = {}^3/_2$ spin-only susceptibilities are observed (cf. Wickman, H. H., et al. *Chem. Phys. Lett.* **1980**, *1*, 117. Wickman, H. H., et al. *Phys. Rev.* **1967**, *155*, 563).

(26) Billing, E.; Williams, R.; Bernal, I.; Waters, J. H.; Gray, H. B. *Inorg. Chem.* **1964**, *3*, 663.

(27) there is evidence that spin relaxation rates can significantly exceed the rates expected from dipolar interaction alone if some small amount of exchange coupling also exists within the system. For example, see: Molin, Y. N.; Salikhov, K. M.; Zamaraen, K. I., "Spin Exchange"; Springer Verlag: Berlin, 1980.

(28) Spartalian, K., unpublished results.

(29) Summerville, D. A.; Cohen, I. A.; Hatano, K.; Scheidt, W. R. *Inorg. Chem.* **1978**, *17*, 2096.

(30) Powers, L., unpublished results.

(31) Shoklnik, G. M.; Geiger, W. E., Jr. *Inorg. Chem.* **1975**, *14*, 313.

(32) Hermann, A. O. Ph.D. Thesis, University of California at Riverside, Riverside, CA, 1972.

(33) Gunter, M. J.; Mander, L. N.; McLaughlin, G. M.; Murray, K. S.; Berry, K. J.; Clark, P. E.; Buckingham, D. A. *J. Am. Chem. Soc.* **1980**, *102*, 1470.

(34) Gunter, M. J.; Mander, L. N.; Murray, K. S.; Clark, P. E. *J. Am. Chem. Soc.* **1981**, *103*, 6784.

(35) Berry, K. J.; Clark, P. E.; Gunter, M. J.; Murray, K. S.; *Nouv. J. Chim.* **1980**, *4*, 581.

(36) Sams, J. R.; Tsin, T. B. In "The Porphyrins"; D. Dolphin, Ed.; Academic Press: New York, **1979**; Vol. IV, 447 .

(37) Carter, K. R.; Antalis, T. M.; Palmer, G.; Ferris, N. S.; Woodruff, W. M. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *78*, 1652.

MCD measurements, have recently proposed the existence of $S = {}^3/_2$ iron in compound c^{37} and some other intermediate oxidation state forms of the oxidase. If the spin of the "silent" iron in the resting oxidase is also assumed to be ${}^3/_2$ instead of ${}^5/_2$, as is normally done, the spin relaxation rate would be expected to be much more rapid. A combination of dipolar coupling and a small amount of exchange coupling between the iron and copper could reasonably produce relaxation rates that broaden both signals past the point of experimental observability.[27] The calculated magnetic moment (assuming the remaining iron and copper are both $S = {}^1/_2$) for such a spin system agrees with experimental values reported for the enzyme[4-6] at least as well as the antiferromagnetically coupled high-spin model.[1,38] The major advantage of this intermediate-spin relaxation model is that it avoids the necessity of requiring an exceptionally and possibly unreasonably large Fe–Cu coupling constant while at the same time adequately explaining both the experimental bulk susceptibility and EPR spectrum.

In summary, the trimeric complex reported here exhibits some unusual and unexpected properties. The observed behavior of the model suggests a description for the spin behavior of the oxidase that is different from the explanation that is most generally accepted. The relative merit of these two alternate explanations should now be subjected to an appropriate degree of critical evaluation based on experimental results obtained from the enzyme.

Acknowledgment. We would like to thank Dr. Linda Powers for providing information on the preliminary EXAFS results and Professor Kevork Spartalian for providing information on the preliminary Mössbauer. We would also like to thank Professor D. B. Brown for helpful discussions on the magnetic susceptibility results and Professor Gareth Eaton for experimental help in obtaining the EPR spectra as well as helpful discussions. This work was supported by the National Institute of Health (GM 26958 and GM 30306).

---

[38] If consideration is given to the possibility of a spin admixture containing a small percentage of $S = {}^5/_2$ iron, the fit can be made arbitrarily as good as desired.

Dependence of the Yield of a Radical-Pair Reaction in the Solid State on Orientation in a Magnetic Field

Steven G. Boxer,* Christopher E. D. Chidsey, and
Mark G. Roelofs

Department of Chemistry, Stanford University
Stanford, California 94305

Received December 7, 1981

We report the observation that the yield of a radical-pair reaction in the solid state can depend strongly on the orientation of the reactants in a magnetic field. This phenomenon is expected to be quite general for radical-pair reactions in rigid environments where the anisotropic magnetic interactions associated with the individual radicals and with the pair maintain fixed values relative to the field during the evolution of the pair spin multiplicity (typically about 1–10 ns). The particular system being investigated is a bacterial photosynthetic reaction center (RC) depleted of secondary acceptors, where a triplet product is formed by radical ion-pair recombination following photoinduced electron transfer. The reactive components in the RC are immobilized in a hydrophobic protein complex.

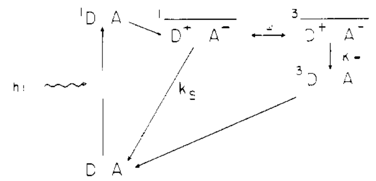


Figure 1. General scheme for photoinduced electron-transfer reactions. In photosynthetic RCs, both D and A are chlorophyll-like chromophores; consequently their radicals are typical aromatic radicals.

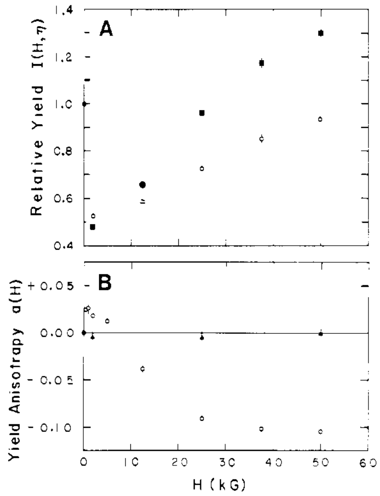


Figure 2. (A) The relative triplet quantum yields, $I(H,\eta)$, for RCs in a viscous buffer: $I(H,0°)$ (○); $I(H,90°)$ (■). The absolute quantum yield at zero field is 0.21.[8] (B) The measured triplet quantum yield anisotropy, $a(H)$ (eq 1) for RCs. $a(H)$ in a viscous buffer (○); $a(H)$ in a nonviscous buffer (▲). Quinone-depleted RCs were suspended in buffer (20mM Tris·HCl, 10 μM EDTA, 0.05% Triton (pH 8.0); nonviscous, 1 cP) or a mixture of this buffer with glycerol (66% glycerol, viscous, ~23 cP), giving a final RC concentration of 10 μM (T = 293 K).

The initial events in photosynthesis following photoexcitation[1] are characteristic of a wide range of electron-transfer reactions (Figure 1). The donor (D) in its first excited singlet state transfers an electron to the acceptor (A) to form a singlet-correlated radical ion pair (RIP). In competition with ion-pair recombination to the ground state (rate constant k_S), the singlet RIP evolves to a triplet RIP, driven by the nuclear hyperfine interactions within each radical and the g factor difference between the radicals. The triplet RIP can then be trapped by the exothermic, spin-allowed recombination reaction to form the molecular triplet ^{3}D, with rate constant k_T. Singlet–triplet mixing is impeded by the triplet splitting due to the isotropic exchange interaction and the anisotropic electron–electron dipolar interaction. The magnitude of the latter depends on the orientation of the RIP in a magnetic field and the distance between the radicals.[2] Likewise, both magnetic interactions which drive singlet–triplet mixing can depend on orientation; the nuclear hyperfine interactions and g factors are each given by their appropriate tensors. Since the contribution of the difference **g** tensor to the rate of singlet–triplet mixing increases linearly with field, whereas the strengths of the electron–electron and electron–nuclear dipolar interactions are field independent, the effects of these anisotropic terms on the triplet quantum yield may vary substantially with the applied field strength, as well as with orientation. Both effects are observed.

Nonoriented quinone-depleted RCs (*R. spheroides*, R-26[3]) were excited essentially isotropically with a subsaturating 8-ns light pulse at 532 nm. The concentration of ^{3}D 3 μs after the flash is conveniently detected with linearly polarized light at 870 nm, where absorption is dominated by the ground state of D. The

---

[1] (a) Rockley, M. G.; Windsor, M. W.; Cogdell, R. J.; Parson, W. W. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2251. (b) Kaufmann, K. J.; Dutton, P. L.; Netzel, T. L.; Leigh, J. S.; Rentzepis, P. M. *Science (Washington, D.C.)* **1975**, *188*, 1301. (c) Holten, D.; Hoganson, C.; Windsor, M. W.; Schenck, C. C.; Parson, W. W.; Migus, A.; Fork, R. L.; Shank, C. V. *Biochim. Biophys. Acta* **1980**, *592*, 461. (d) Thurnauer, M. C.; Katz, J. J.; Norris, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 3270. (e) Bowman, M. K.; Budil, D. E.; Closs, G. L.; Kostka, A. G.; Wraight, C. A.; Norris, J. R. *Ibid.* **1981**, *78*, 3305.

[2] A more detailed analysis of the dipolar interaction in the primary RIP of RCs is given in: Roelofs, M. G.; Chidsey, C. E. D.; Boxer, S. G. *Chem. Phys. Lett.* **87**, 582 (1982).

[3] Butler, W. F.; Johnston, D. C.; Shore, H. B.; Fredkin, D. R.; Okamura, M. Y.; Feher, G. *Biophys. J.* **1980**, *32*, 967.