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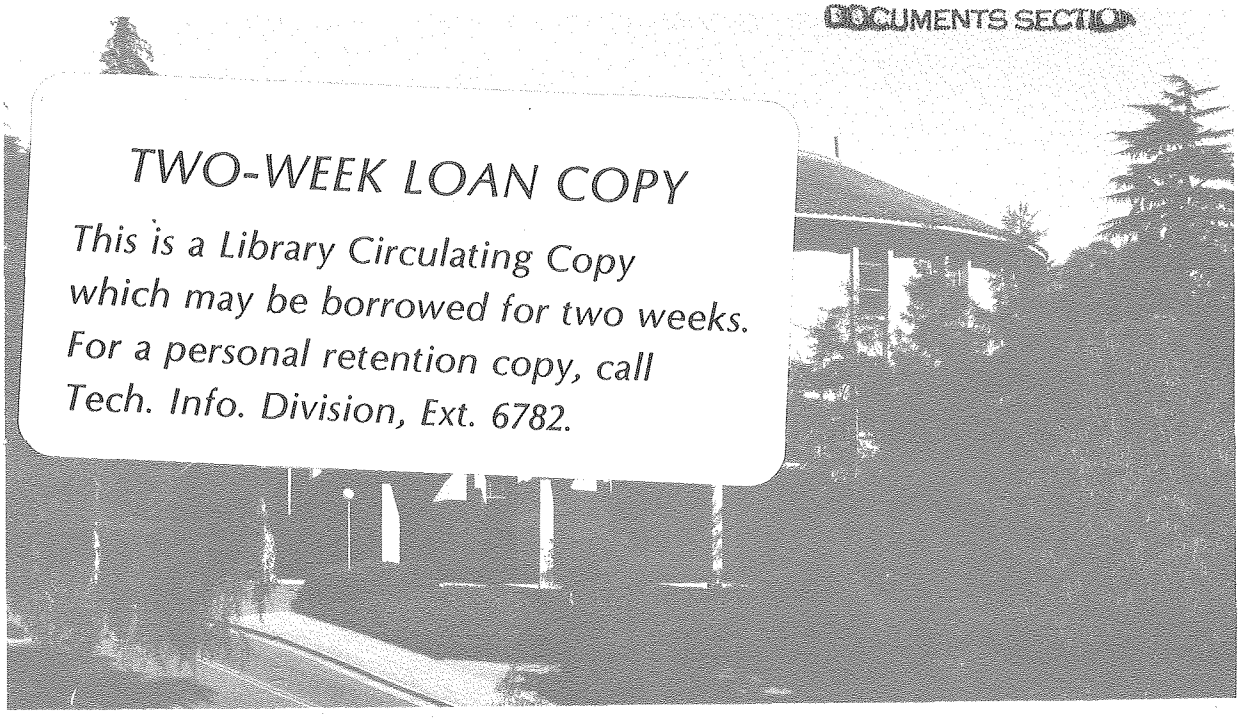

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DISCLAIMER

CORRELATION BETWEEN THE OPTICAL AND MAGNETIC PROPERITES OF FERRIC N-ACETYLATED HEME OCTAPEPTIDE COMPLEXES*

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ABSTRACT

The room temperature magnetic susceptibility of the complexes of the ferric N-acetylated heme octapeptide (N-H8PT) from horse heart cytochrome c is known to be generally consistent with the absorption and magnetic circular dichroism (MCD) spectra of these complexes. However, the N-acetylated methionine complex of the N-H8PT, which has axial coordination identical to that of the parent molecule, is found to exhibit a thermal mixture of high spin (S=5/2) and low spin (S=1/2) states. The temperature dependence of the magnetic susceptibility of the N-acetylmethionine complex yields $\Delta H°$ = -7.6kcal/mole and $\Delta S°$ = -25.9 e.u. for a high to low spin transition. The electron spin resonance (ESR) spectrum of the N-acetylmethionine complex indicates a low spin ground state, with g values at 1.51, 2.31, and 2.91, which are distinct from those of cytochrome c. The axial (Δ) and rhombic (V) distortion parameters of the $^2T_{2g}$ state correspond to 2.96λ and 1.94λ, respectively, where λ is the spin-orbit coupling constant. A model is proposed to account for the uniqueness of the N-acetylmethionine complex: a change in the Fe-S distance may play a role in regulating the redox properties of cytochrome c.

INTRODUCTION

In hemoproteins the magnetic properties of iron are known to be closely related to the electronic structure of the porphyrin, owing to extensive mixing of iron d orbitals with porphyrin π orbitals via spin-orbit interaction (1-3). This has been demonstrated nicely in a large number of hemoprotein derivatives (4-14). Our investigation of the absorption and MCD spectral behavior and of the magnetic susceptibility of the ferric N-H8PT complexes has shown that such correlation may be extended to model system studies (15). The most peculiar observation, however, was the optical and magnetic susceptibility evidence for the coexistence of high and low spin states in a model system of cytochrome c, the N-acetylmethionine complex of the N-H8PT. The presence of thermal spin equilibrium between the two spin states at ambient temperature is common in ferric hemoprotein derivatives (16-18), although optical and/or magnetic susceptibility evidence alone cannot distinguish it from thermal mixing of the two chemically distinct species, one with a high spin and the other with a low spin ground state, each exhibiting no thermal spin equilibrium, or from quantum mechanical admixing of the spin states (19,20).

For the N-acetylmethionine complex we sought to verify the spectral observation by measurement of the temperature dependence of the magnetic susceptibility and the low temperature ESR spectrum. The amount of low spin state estimated under the assumption of thermal spin equilibrium is consistent with the absorption evidence. The thermodynamic parameters involved in the spin transition are characterized. The low temperature ESR spectrum of the N-acetylmethionine complex is shown to reflect a single low spin species with g values different from those of cytochrome c. The

possible role of this electronic structural difference in the regulation of the redox properties of cytochrome c is discussed.

MATERIALS AND METHODS

Magnetic susceptibility was measured by the NMR method of Evans (15,21). NMR measurements were carried out on a Varian A-60 NMR spectrometer with a V-6040 variable temperature controller. The temperature dependence of the probe was monitored by the separation of the methyl and hydroxyl proton resonance lines of methanol.

ESR spectra were recorded on a Varian E-9 ESR spectrometer in the X-band region with an Air Products liquid helium cryostat.

RESULTS

Table I presents a summary of the effective magnetic moments, μ_{eff}, and estimated % low spin of the N-H8PT complexes at pH 7.0, 25°C (15). The value of Δf indicates typical frequency shifts in Hz of the acetone proton resonance line arising from the total susceptibility: χ_M^{corr} represents the molar susceptibility after correcting for the diamagnetic contribution of the N-H8PT, which is calculated to be -742×10^{-6} erg $G^{-2}M^{-1}$ using Pascal's constants (22). The percentage of the low spin state is obtained as described by Iizuka, et al. (17). Of particular interest are the values of μ_{eff} for both fluoride and aquo complexes; they are less than the anticipated high spin value of 5.9 μ_B. In the case of the aquo complex, the decreased magnetic moment has been interpreted as arising from weak antiferromagnetic coupling via π donor-acceptor type interactions, which is consistent with the absorption behavior at high heme concentration (15,24,25). The decreased magnetic moment of the fluoride complex is most likely due to the same

effect. The absorption spectra have shown that fluoride might not be a strong enough ligand to break all of the π donor-acceptor type interactions at high heme concentrations (15).

The N-acetylmethione complex exhibits μ_{eff} 4.0 μ_B, much larger than the expected low spin value of native cytochrome c (Table I). In fact, the corresponding % low spin for the N-acetylmethionine complex agrees well with the optical evidence, where the intensity of the 625 nm charge transfer band indicates the presence of about 35% high spin state (15). Fig. 1 illustrates our interpretation of the temperature dependence of μ^2_{eff} of the N-acetylmethionine complex between -5 and 40°C. A linear dependence is observed between lnK and 1/T, where K is the equilibrium constant for a high spin to low spin transition. $\Delta H°$ and $\Delta S°$ for such a transition are found to be -8.0 Kcal/mole and -25.2 e.u., respectively. In our previous study (15), however, about 10% of the N-H8PT was shown to be ligand unbound at 2.5M N-acetylmethionine concentration. Thus, thermodynamic contributions from the temperature-dependent ligand binding need to be taken into account. From the temperature dependence of the equilibrium quotient for N-acetylmethionine binding determined spectrally, the corresponding $\Delta H°$ and $\Delta S°$ were shown to be -0.44 Kcal/mole and 0.74 e.u. (15).

Fig. 2 shows the ESR spectra of the ferric H8PT, N-H8PT, N-acetylmethionine complex and of cytochrome c at 15°K. The spectrum of the aggregated H8PT (top) indicates the presence of both high and low spin signals. The low-spin signal exhibits g values at 1.83, 2.13 and 3.17 and is very broad. The broadened low spin type spectrum has been observed previously for the aggregated heme undecapeptide, with g values at 1.46, 2.16 and 3.03 (26). Such band broadening appears to reflect heme-heme interactions. The ESR spectrum of the aquo complex of the N-H8PT indicates

the presence of a major high spin component with $g_{\perp} = 6.03$ and $g_{\parallel} = 2.01$ and a minor low spin component with $g_y = 2.28$ and $g_z = 2.96$ (g_x not resolved). The low spin component is due to the hydroxide form of the N-H8PT. The N-H8PT at pH 11.5 yields g values at 1.93, 2.28 and 2.95. This indicates that the decreased magnetic moment of the N-H8PT is in part due to the hydroxide form. The ESR spectrum of the N-acetylmethionine complex indicates a low spin ground state which has g-values that are distinctly different from those of cytochrome c. A minor high spin component corresponds to the aquo form of the N-H8PT. The estimated amount is 12% by direct comparison of the doubly integrated area of the $g_{\perp}$ signal, using the aquo complex of the N-H8PT of a known concentration as a standard. This is consistent with the results of optical absorption studies (15).

DISCUSSION

We have demonstrated that there is a good correlation between the electronic structure of porphyrins and the magnetic properties of iron in the ferric N-H8PT complexes (15). The measurement of the temperature dependence of the magnetic susceptibility of the N-acetylmethionine complex shown in Fig. 1 confirms the presence of thermal spin equilibrium at room temperature. The behavior of the N-acetylmethionine complex is atypical in every case examined.

According to Iizuka (17), in a high spin to low spin transition, $\Delta H°$ corresponds to the energy difference between the two spin states while $\Delta S°$ corresponds largely to the changes in the degrees of freedom associated with spin degeneracy change. From the $\Delta H°$ of -7.6 Kcal/mole (corrected for the thermodynamic contribution due to N-acetylmethionine binding), the energy difference is calculated to be $2638 cm^{-1}$. The positive sign is consistent

with the low-spin ground state. Its magnitude, however, is apparently much greater than kT at room temperature. In fact, most derivatives of ferrimyoglobin, hemoglobin and peroxidase are known to exhibit large energy differences and still show thermal spin equilibrium (16-18). This may be realizable only when the entropy term is large enough to overcome the large enthalpy change, such that $\Delta G^\circ = 0$ occurs near room temperature. Surprisingly, this is the case even in the N-acetylmethionine complex, suggesting that the entropic driving force must be an intrinsic property of the heme coordination and/or its association with the solvent medium.

The azide complex has been demonstrated also to exhibit a thermally-mixed spin state, with $\Delta H^\circ = -3.89$ Kcal/mole and $\Delta S^\circ = -8.6$ e.u. (27). It is important to note that both imidazole and azide complexes of cytochrome c do not give rise to significant thermal spin equilibrium between 20 and 30°K (28). In the model complex the heme is totally exposed to the aqueous medium and is free of van der Waals contacts with the protein, whereas in cytochrome c the heme group is confined to a particular environment. In the former case we may expect a larger change in the number of degrees of freedom associated with the spin transition. Perhaps only hemes with a high spin ground state are capable of exhibiting thermal mixing of spins upon ligand binding.

The uniqueness of the N-acetylmethionine complex is also evident in the ESR spectrum. It exhibits low-spin g values that are different from those of native cytochrome c (Fig. 2). The anisotropy of low-spin heme g tensors arises from spin-orbit coupling that mixes the upper Kramers doublets into the lowest Kramers doublet of the $^2T_{2g}$ state in D_{2h} symmetry (29,30). The ground state wavefunction therefore reflects the magnitude of energy separation among the three d orbitals involved in ^2T_2g. Following Taylor's calculation (30), the axial (Δ) and rhombic (V) distortion parameters for

cytochrome c are found to be 1020 cm^{-1} and 600 cm^{-1}, respectively, assuming that the spin-orbit coupling constant λ is 400 cm^{-1} for a d^5 system. These values are somewhat different from the results reported by Blumberg (31). Fig. 3 shows the energy separations and symmetry assignments of the three Kramers doublets of the N-acetylmethionine complex compared with those of cytochrome c. Both the axial and rhombic splittings are greater in the N-acetylmethionine complex. While the axial distortion reflects the effective crystal field strength determined by the electron density at the iron, the rhombic distortion reflects the geometry of the bound ligand with respect to the heme plane, i.e., the extent of π interaction between the ligand and heme. In view of this, we propose a model in which the Fe-S distance is different in the N-methionine complex from that in cytochrome c. Because the greater axial distortion indicates stronger orbital overlap between the axial ligands and iron, if we assume that the Fe-N distance remains constant, then shortening of the Fe-S bond length would account for the observed effect. In addition, if there is a stronger orbital overlap, the ferric ion form will be stabilized owing to greater electron density at the iron, which predicts a lower midpoint potential, E_m, for the N-acetylmethionine complex. Indeed, the E_m of horse heart cytochrome c is +250 mv while that of the N-acetylmethionine complex is -50 mv (32). Our model is consistent with the view proposed by Williams (33) in which shortening of the Fe-S bond by 0.1Å is expected to decrease the E_m by 400 mv. From these results, we believe that one of the ways of controlling a wide variation of midpoint potential in heme proteins is via changes in the extent of axial ligand and iron interaction and in the geometry of the bound axial ligands modulated by the protein.

In summary, our model system has shown that [1] the ligand field strength of the N-acetylmethionine ligand is "strong", as expected from the behavior of cytochrome c; [2] observation of thermal spin equilibrium in the N-acetylmethionine complex is purely entropic in origin and not favored energetically; and [3] the protein structure of heme proteins appears to provide a fine control for obtaining an effective ligand field environment and altered geometry of the bound axial ligands, which may in turn affect the redox properties.

REFERENCES

1. M. Gouterman, J. Chem. Phys. 30, 1139 (1959).

2. M. Kotani, Adv. Chem. Phys. 7, 159 (1964).

3. C. Weiss, H. Kobayashi and M. Gouterman, J. Mol. Spectr. 16, 415 (1965).

4. A. S. Brill and R.J.P. Williams, Biochem. J. 78, 246 (1961).

5. J. Bettlestone and P. George, Biochemistry 3, 707 (1964).

6. P. George, J. Bettlestone and J.S. Griffith, Revs. Mod. Phys. 36, 441 (1964).

7. D.W. Smith and R.J.P. Williams, Biochem. J. 110, 297 (1968).

8. D.W. Smith and R.J.P. Williams, Structure and Bonding 7, 1 (1970).

9. B. Briat, D. Berger and M. Leliboux, J. Chem. Phys. 57, 5606 (1972).

10. A.M. Arutjunjan, A.A. Konstantinov and Y.A. Sharonov, FEBS Letters 46, 317 (1974).

11. J.I. Treu and J.J. Hopfield, J. Chem. Phys. 63, 613 (1975).

12. L.E. Vickery, in Proc. First Taniguchi Int. Symp. Biophys., Metallo-Protein Studies Utilizing Paramagnetic Effect of the Metal Ions as Probes. M. Kotani and A. Tasaki, Eds., Osaka University, Japan, pp. 27-50.

13. L.E. Vickery, T. Nozawa and K. Sauer, J. Am. Chem. Soc. 98, 351 (1976).

14. M.A. Livshitz, A.M. Arutyunyan and Y.A. Sharonov, J. Chem. Phys. 64, 1276 (1976).

15. E.K. Yang, L.E. Vickery and K. Sauer, in preparation.

16. T. Iizuka, M. Kotani and T. Yonetani, Biochim. Biophys. Acta, 167, 257 (1968).

17. T. Iizuka and M. Kotani, Biochim. Biophys. Acta, 181, 275 (1969).

18. T. Iizuka and T. Yonetani, Adv. Biophys. 1, 157 (1970).

19. M.M. Maltempo, J. Chem. Phys. 61, 2540 (1974).

20. M.M. Maltempo and T.H. Moss, Quarterly Review of Biophysics, 9, 181 (1976).

21. D.F. Evans, J. Chem. Soc. 2003, (1959).

22. P.W. Selwood, "Magnetochemistry", 2nd Ed., Interscience Publishers, New York 1956. p. 91.

23. A.M.T. Jehanli, D.A. Stotter and M.T. Wilson, Eur. J. Biochem. 71, 613 (1976).

24. H.J. Schugar, G.R. Rossman and H.B. Gray, J. Am. Chem. Soc. 91, 4564 (1969).

25. H. Goff and L.O. Morgan, Inorganic Chemistry, 15, 2069 (1976).

26. D.V. DerVartanian, Biochim. Biophys. Res. Comm. 41, 932 (1970).

27. Y.-P. Huang and R.J. Kassner, J. Am. Chem. Soc. 101, 5807 (1979).

28. M. Ikeda-Saito and T. Iizuka, Biochim. Biophys. Acta, 393, 335 (1975).

29. G.M. Harris Loew, Biophys. J. 10, 196 (1970).

30. C.P.S. Taylor, Biochim. Biophys. Acta, 491, 137 (1977).

31. W.E. Blumberg and J. Peisach, in "Probes of Structure and Function of Macromolecules and Membranes", vol. II, B. Chance, T. Yonetani and A.S. Mildvan, Eds., Academic Press, New York, 1971, pp. 215-228.

32. H.A. Harbury, J.R. Cronin, M.W. Fanger, T.P. Hettinger, A.J. Murphy, Y.P. Myer and S.N. Vinogradov, Proc. Nat. Acad. Sci., 54, 1658 (1965).

33. G.R. Moore and R.J.P. Williams, FEBS Letters, 79, 229 (1977).

LEGENDS

Figure 1. A plot of ln K versus 1/T between -5 and 40°C for a high spin to low spin transition in the N-acetylmethionine complex of N-H8PT as determined by the NMR method.

Figure 2. The electron spin resonance spectra of the aggregated H8PT, N-acetylated H8PT, N-acetylmethionine complex of N-H8PT and cytochrome c in a ferric state. Microwave frequency, 9.26 G Hz; microwave power, 10 mW; modulation amplitude, 10 G and temperature, 15°K.

Figure 3. The energy splittings of the axial and rhombic distortions in the $^{2}T_{2g}$ set for cytochrome c and the N-acetylmethionine complex of N-H8PT as calculated from the anisotropy of the g values in Fig. 2 and a spin-orbit coupling constant, λ, of 400 cm^{-1}.

TABLE I

MAGNETIC SUSCEPTIBILITY OF Fe (III) - N-H8PT COMPLEXES MEASURED BY NMR AT 25°C

Δf, frequency shift in Hz; χ_M^{corr}, molar susceptibility in erg $G^{-2}M^{-1}$, corrected for the diamagnetic contribution; μ_{eff}, effective magnetic moment in Bohr magnetons

Ligand	Heme Conc. (mM)	Δf	$\chi_M^{Corr} \times 10^3$	μ_{eff}	% Low Spin
Fluoride	1.84	2.75	12.62	5.48	16
Water	1.85	2.70	12.34	5.42	19
Hydroxide	1.49	1.30	7.67	4.28	56
Azide	2.37	1.30	5.10	3.49	76
N-Methionine	2.11	1.60	6.79	4.02	63
Cyanide	1.82	0.60	3.35	2.83	90
Imidazole	1.72	0.40	2.58	2.48	96

XBL 786 - 4011

The data are taken from Ref. 15.

THERMODYNAMICS OF SPIN TRANSITION IN Fe(III)-N-H8PT-N-METHIONINE COMPLEX

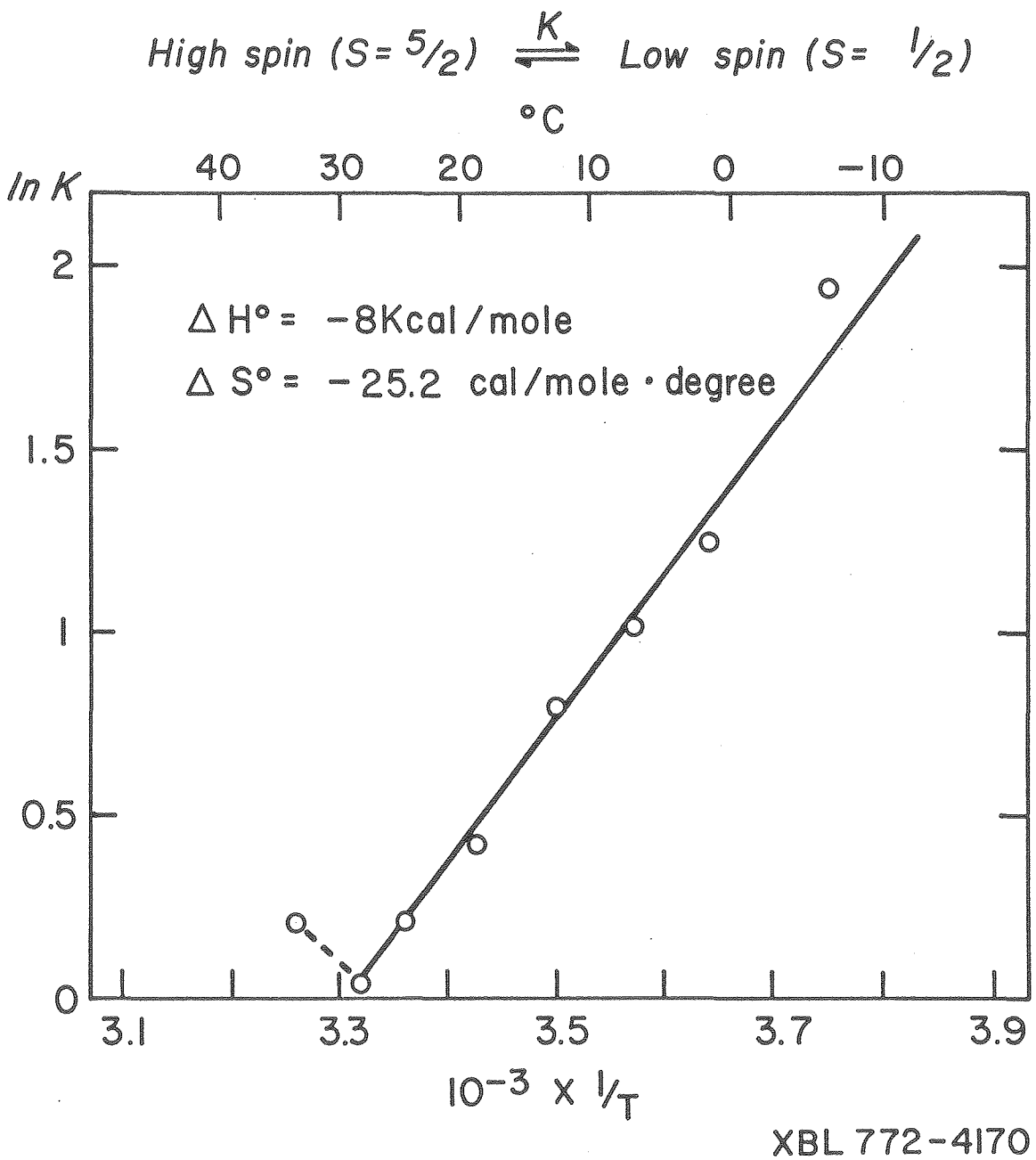

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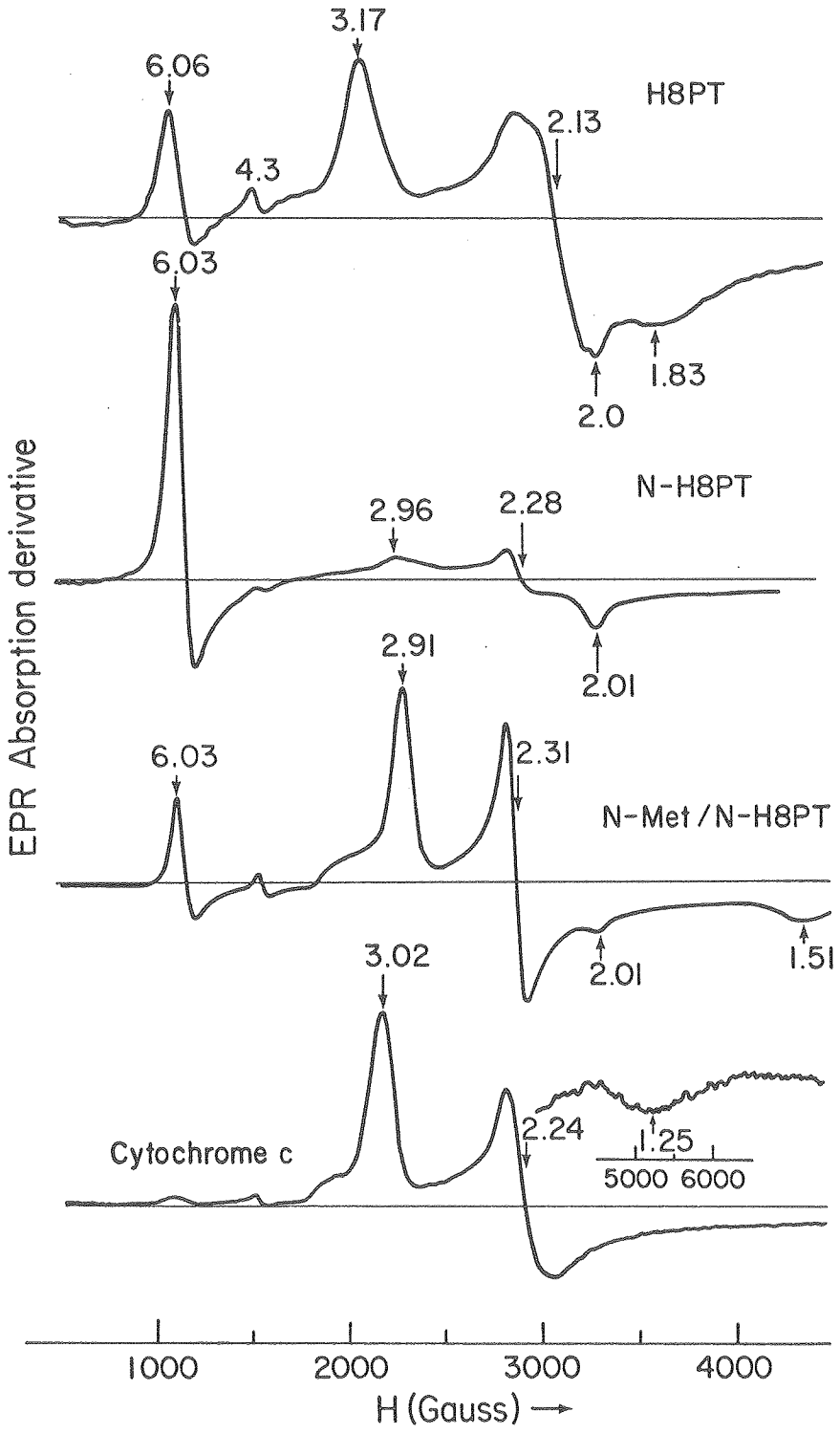
Fig. 1



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Yang & Sauer
Fig. 2

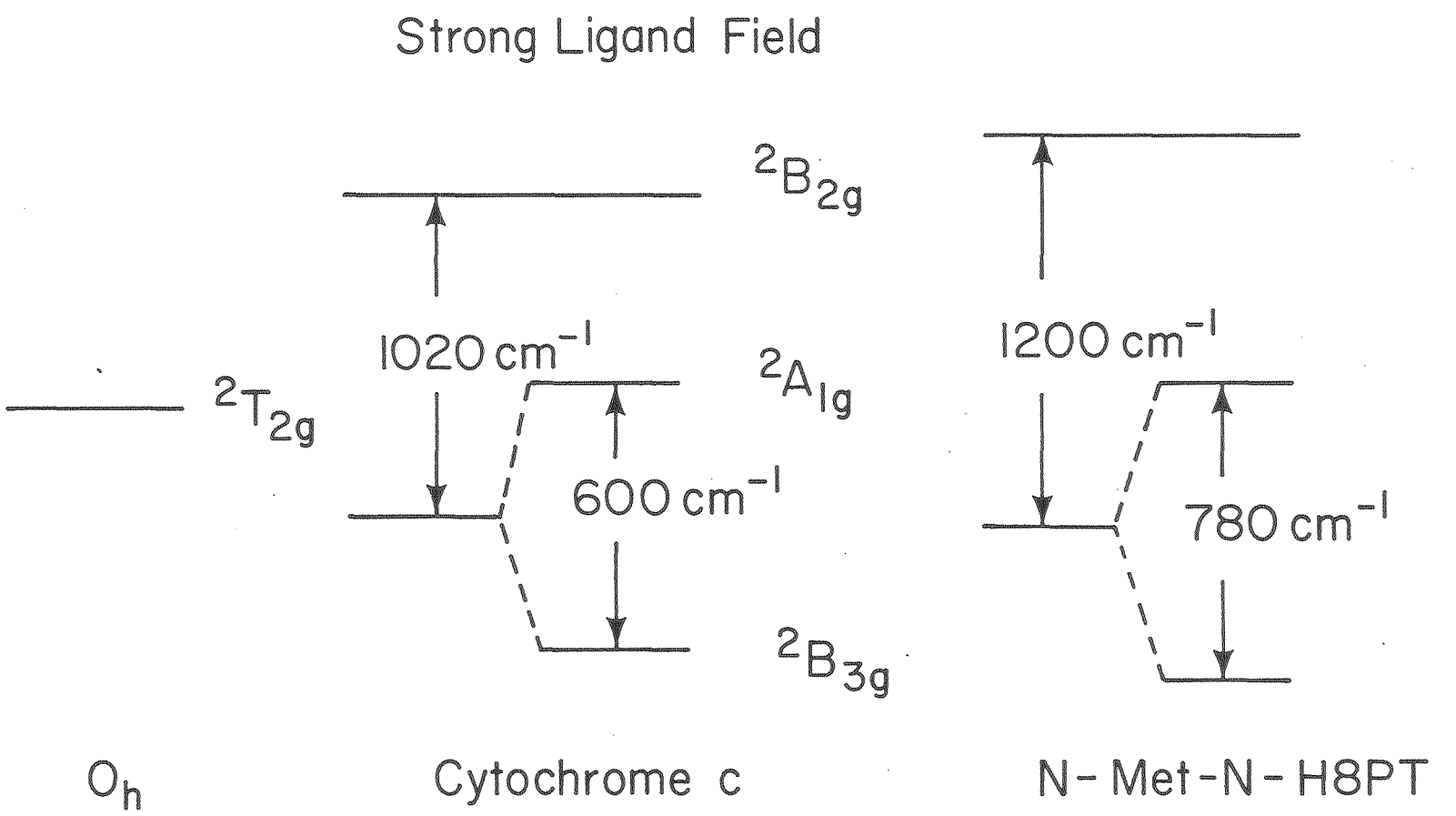

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Fig. 3