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ARTICLE

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Analysis of the paramagnetic shifts of haem carbon resonances in bovine ferricytochrome b_5

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Abstract Recently published chemical shifts for haem ^{13}C nuclei in bovine ferricytochrome b_5 (Lee KB, Kweon J, Park H (1995) Assignment of hyperfine-shifted heme carbon resonances in ferricytochrome b_5. FEBS Lett. 367: 77–80) are analysed in terms of haem molecular orbitals with perturbed D_{4h} symmetry. Since a crystal structure of this protein is available, together with extensive ^{1}H assignments both in the oxidised and reduced forms, the paramagnetic shifts can be separated into dipolar and Fermi contact contributions by using an empirical magnetic susceptibility tensor. The results are compared with the orientation of the tensor and the geometry of the haem ligands. This comparison casts doubt on one of the ^{13}C assignments and demonstrates that the asymmetry of the haem electronic structure is dominated by the influence of both of the His ligands. The ^{13}C chemical shifts of two haem methyl groups in the minor form of the protein, in which the haem is approximately rotated by 180° about its 5CH-15CH axis, are also evaluated.

Key words Cytochrome b_5 · ^{13}C NMR · Fermi contact shift · Heme electronic structure · Ligand orientation · Heme rotation

Introduction

Attempts to understand the factors which control electronic structure in haem proteins have a long history which involves much elegant work, both theoretical and experimental. The magnetic properties of low spin Fe(III) in a perturbed cubic environment are well understood (Griffith 1957; Harris 1966; Oosterhuis and Lang 1969) and the theories have been tested extensively by single crystal EPR studies of model compounds (Walker et al. 1986; Byrn et al. 1993). These studies demonstrated that the orientation of the two axial ligands dominates both the magnitude and the orientation of the rhombic perturbation of the ligand field in model compounds. Extending this understanding to the electronic properties of the porphyrin involves the highest occupied π molecular orbitals, which occur as a degenerate pair under D_{4h} symmetry (Longuet-Higgins et al. 1950; Shulman et al. 1971), and the unpaired electron distribution is most easily probed by NMR studies of haem complexes in solution (e. g. La Mar and Walker 1979). Unfortunately, studies of model compounds in solution are often complicated by lability of the axial ligands and consequent uncertainty over their orientation and, although asymmetric haem subtituents may be incorporated into model compounds (La Mar et al. 1978) and ligand rotation may be hindered (Traylor and Berzinis 1979), they cannot resolve questions about the influence of a surrounding polypeptide. On the other hand, studies of haem proteins themselves are complicated by experimental difficulties. The early attempt by Shulman et al. (1971) to rationalise the chemical shifts of the haem methyl resonances in metcyanomyoglobin in terms of the π molecular orbitals succeeded in establishing the utility of the model but could not establish a firm relationship between the orientation of the rhombic perturbation and that of the axial His ligand, largely because of the limited data then available. More recently, extensive assignments in the proton NMR spectra of proteins for which crystal structures are available have made it possible to determine the orientation of the magnetic axes by fitting paramagnetic shifts to a metal centred dipolar field (Kurland and McGarvey 1970; Keller and Wüthrich 1972). Such information should have been sufficient to establish that the conclusions reached about the relationship between the axial ligands and the rhombic perturbation in single crystal EPR studies of model compounds are also applicable to haem proteins. However, doubts remained because of a persisting confusion over the relationship between the orientation of the rhombic perturbation and the magnetic axes (Shulman et al. 1971) and led to the conclusion that the electronic structure of ferricy-

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tochrome b_5 is determined primarily by just one of the two axial His ligands (McLachlan et al. 1988). Direct support for the "dominant role" of His 39 was also inferred from the large shifts of the His 39 βCH_2 protons (Lee et al. 1994), although this appears to be a simple geometric effect which bears no relation to the strength of the His-Fe bond.

There is a fundamental problem with proton NMR insofar as the resonances of haem substituents cannot provide an accurate measure of the unpaired electron distribution in the haem molecular orbitals with the present level of understanding. The paramagnetic shifts have comparable contributions from Fermi contact shifts and dipolar shifts: the dipolar shifts may be calculated to a good approximation once the magnetic susceptibility tensor is known (Kurland and McGarvey 1970) but the hyperfine coupling constants which determine the Fermi contact shifts are in fact quite variable and may be affected by a number of geometric and electrostatic factors (Karplus and Fraenkel 1961). Despite the experimental difficulties, ^{13}C NMR has considerable advantages since the Fermi contact shifts are generally much larger than the dipolar shifts and the hyperfine coupling constants appear to be reasonably constant for nuclei positioned α to the haem (Wüthrich and Baumann 1973). Recently, analysis of ^{13}C NMR data from several haem proteins has established an excellent correlation between the orientations of axial ligands and the orientation of the rhombic perturbation which dominates the form of the π molecular orbitals, and hence to the orientation of the magnetic axes (Turner 1995; Turner et al. 1995; Banci et al. 1995).

Thus it is clear that the factors which determine the magnetic properties of low spin haems in proteins in solution are essentially similar to those for haem complexes in crystals and that this is consistent with the form of the molecular orbitals. It should now be possible to probe more subtle effects involving a variety of axial ligands and asymmetric haem substituents, and the influence of the surrounding protein, as well as seeking to understand the variability of proton hyperfine coupling constants. For this purpose, we need to compare haem proteins which have structures which are known to high resolution, extensive NMR assignments in the oxidised and reduced forms for the empirical determination of magnetic susceptibility tensors, and complete assignments of the ^{13}C NMR resonances of the haem substituents. Bovine cytochrome b_5 is one of the few proteins containing haem b for which the first two of these requirements are met, and now several ^{13}C assignments have become available. In a recent article, Lee et al. (1995) made use of heteronuclear multiple quantum coherence (HMQC) experiments to assign ^{13}C NMR signals from haem substituents in bovine ferricytochrome b_5. Although the new assignments (Lee et al. 1995) are quite similar to those obtained from rat ferricytochrome b_5 (Banci et al. 1994; Banci et al. 1995), their analysis is of interest since there is a crystal structure available for bovine cytochrome b_5 (Mathews and Durley 1991) but not for the protein from rat, and so the relationships established by the model can now be tested more precisely. This analysis also provides a test of the assignments and further evidence for the proposal that the minor B form of the protein, which comprises 40% of cytochrome b_5 from rat but only 10% of the bovine protein, is related to the major A form by a rotation of the haem b through 180° about the 5CH-15CH axis with little change in the structure of the surrounding protein (Keller and Wüthrich 1980; Mathews 1980).

Analysis of ^{13}C shifts

NMR shifts of the haem substituents in paramagnetic molecules are the sum of a diamagnetic and a paramagnetic contribution. The latter may be separated into Fermi contact and dipolar contributions once the magnetic susceptibility tensor of the protein is known (Keller and Wüthrich 1972). In the case of bovine ferricytochrome b_5, the tensor has been determined by fitting the dipolar shifts of a large number of specifically assigned protons (Guiles et al. 1990; Veitch et al. 1990; Banci et al. 1995) with atomic coordinates obtained from the crystal structure (Mathews and Durley 1991) so that the dipolar shifts of the haem nuclei can be calculated and separated from the Fermi contact contributions to a very good approximation. The Fermi contact shifts of ^{13}C nuclei positioned α to the haem, δ_{FCi}, are dominated by polarisation of the carbon s electrons by unpaired electron density, ρ_i in the π orbital of the neighbouring pyrrole β carbon (Karplus and Fraenkel 1961):

$$\delta_{FCi}\,(\text{ppm}) = 10^6 \cdot 2\pi\, g_e \beta_e\, S(S+1)\, Q\, \rho_i/(3\, kT\, \gamma_C) \qquad \text{(Eq. 1)}$$

in which symbols have their usual meanings and Q is a constant to be determined. In addition, the vinyl carbons of haems b experience shifts caused by delocalisation of unpaired electrons from the π molecular orbital of the haem. A theoretical treatment of the shifts of the vinyl α and β carbons showed that the contribution of delocalisation to the isotropic shift of the α carbon is approximately equal to –0.28 times the total shift observed for the vinyl β carbon and that the factor is independent of the vinyl orientation (Banci et al. 1995), but that treatment took no account of the effects of hyperconjugation or of ligand centred dipolar shifts (Kurland and McGarvey 1970). An empirical comparison of the vinyl α and β carbon shifts in several low spin complexes of haem b (Timkovich 1991; Banci et al. 1994; Banci et al. 1995) yielded a slightly larger factor of –0.42 (Pierattelli et al. 1996), which we shall use here.

The set of Fermi contact shifts of ^{13}C nuclei positioned α to the haem, corrected for the effect of delocalisation onto the vinyl groups, then provides a sensitive measure of the unpaired π electron density on the pyrrole β carbons, ρ_i. This distribution may be modelled by taking the highest partially occupied π molecular orbitals in an idealised haem with D_{4h} symmetry, which we label ϕ_x and ϕ_y, and applying a rhombic perturbation (Longuet-Higgins et al. 1950; Oosterhuis and Lang 1969; Shulman et al. 1971). The unperturbed orbitals form a degenerate pair in which the π electron density on the pyrrole β carbons is determined by just two molecular orbital coefficients, c_1 and c_5 (Longuet-Higgins et al. 1950), which appear to show little variation among haem proteins and so we shall use the values obtained for horse cytochrome *c* ($c_1 = 0.1360$, $c_5 = 0.0661$) (Turner 1995). The rhombic perturbation determines the orientation of the modified π molecular orbitals

$$|\Phi_x\rangle = \cos\theta\,|\phi_x\rangle + \sin\theta\,|\phi_y\rangle; \quad |\Phi_y\rangle = \cos\theta\,|\phi_y\rangle - \sin\theta\,|\phi_x\rangle \qquad \text{(Eq. 2)}$$

and also the energy separation between them (Oosterhuis and Lang 1969; Walker et al. 1986). Finally, we assume that the unpaired electron density in each of the perturbed orbitals has a simple Boltzmann distribution. Thus the set of eight Fermi contact shifts from the haem substituents may be fitted with only three parameters: the orientation of the rhombic perturbation in the plane of the haem, the magnitude of the perturbation, and the degree of polarisation induced by the π electron density on the pyrrole β carbons.

The orientation of the rhombic perturbation, which is equivalent to the mixing parameter for the unperturbed orbitals, θ in Eq. 2, is related to the orientation of largest component of the magnetic susceptibility tensor in the plane of the haem (usually labelled χ_y) by rotation in the opposite sense with respect to the bonds between the haem iron and pyrrole nitrogens (Oosterhuis and Lang 1969). An excellent correlation between the direction of the perturbation and the orientation of the axial ligands has been found in several haem proteins (Turner 1995; Turner et al. 1995; Banci et al. 1995) and the magnitude of the perturbation, represented by the energy separation of the perturbed π molecular orbitals, ΔE, has also been found to correlate with the relative orientations of the axial ligands in several bis-Histidine haem proteins (Turner et al. 1995; Banci et al. 1995). The third parameter, the factor representing the polarisation of the *s* orbitals of the substituent carbons, Q in Eq. 1, has been calculated to be –39 MHz (Karplus and Fraenkel 1961), but empirical determinations have yielded values close to –30 MHz (Strom et al. 1972).

Table 1 Observed and calculated ^{13}C shifts (ppm) for haem substituents in the A form of bovine ferricytochrome b_5 at 298 K. The measured shifts (δ_{obs}) are from Lee et al. (1995), the diamagnetic reference (δ_{dia}) were collected in Banci et al. (1995), and the dipolar shifts (δ_{pc}) were calculated as described in the text. The contribution from Fermi contact shifts, with corrections for conjugation of the vinyl groups marked (*), is labelled δ_{FCobs}, and the result of fitting these shifts to the model described in the text (ignoring the assignment of the 8^1CH vinyl, for which values are given in parenthesis) is labelled δ_{FCcalc}. The parameters obtained for the orbital mixing, energy separation, and polarisation factor are 68.9°, –4.02 kJ mol^{-1}, and –31.15 MHz. The final column gives predictions for the chemical shifts of the B form of the protein made by fitting the 7^1CH_3 shift at –61 ppm and the 18^1CH_3 shift at –45 ppm by varying only the orbital mixing parameter (found to be 8.3°) with the other parameters fixed at the values found for the A form.

	A form					B form
Group	δ_{obs}	δ_{dia}	δ_{pc}	δ_{FCobs}	δ_{FCcalc}	δ
2^1CH_3	–16.6	12.1	–1.84	–26.86	–35.70	–11.8
3^1CH	20.6	132.9	–5.08	–61.97*	–62.43	75.6
3^2CH_2	222.3	117.1	–2.53	107.73	–	–
7^1CH_3	–33.3	12.1	–7.83	–37.57	–39.23	–48.5
8^1CH	(59.0)	132.9	–4.75	(–63.64)*	–12.50	56.3
8^2CH_2	127.0	117.1	–3.22	13.12	–	–
12^1CH_3	–34.8	12.1	–1.73	–45.17	–35.70	–11.7
13^1CH_2	–44.5	22.8	–5.05	–62.25	–62.43	–13.5
17^1CH_2	–26.2	22.8	–7.48	–41.52	–39.23	–37.8
18^1CH_3	–4.6	12.1	–4.48	–12.22	–12.50	–36.3

Results and discussion

The ^{13}C chemical shifts of haem substituents in the A form of bovine ferricytochrome b_5 tabulated by Lee et al. (1995) are compared with diamagnetic reference shifts (Banci et al. 1995) in Table 1. The ^{13}C dipolar shifts listed in Table 1 were calculated according to the standard expression (Bertini and Luchinat 1986) using the magnetic susceptibility tensor derived from proton measurements made at 303 K (Veitch et al. 1990; Banci et al. 1995), with a simple Curie law correction to 298 K, and atomic coordinates from the crystal structure (Mathews and Durley 1991). The Fermi contact shifts obtained by subtracting the diamagnetic and the dipolar shifts from the total paramagnetic shifts are also listed for comparison with the calculated values. Note that the shifts of the vinyl α carbons are corrected for delocalisation of unpaired electron from the pyrroles onto the vinyl groups by subtracting –0.42 times the shifts of the vinyl β carbons, as discussed above.

The Fermi contact shifts of the eight carbons positioned α to the haem were then fitted to the model of π molecular orbitals with perturbed D_{4h} symmetry as described in the previous section. The shift reported for the 8^1CH vinyl α carbon clearly does not fit the model and so it was excluded from the calculation, the results of which are included in Table 1, and the quality of the fit may also be judged from the schematic representation shown in Fig. 1. The calculation predicts that the 8^1CH carbon should have a Fermi contact shift of –12.5 ppm induced by unpaired electron density on the neighbouring pyrrole β carbon and hence, by reversing the analysis, that the resonance should be observed at 110 ppm. There is in fact a small signal which can be seen in Fig. 1 of Lee et al. (1995) close to the predicted ^{13}C shift and at about 4.8 ppm in the ^1H dimension. Cross peaks with ^1H frequencies in this region are likely to be weak and distorted as a result of presaturation of the residual $^1H^2HO$ signal and it would be necessary to examine spectra obtained at other temperatures before confirming this revision.

The parameters obtained from the model are quite similar to those obtained for the A form of rat ferricytochrome b_5 (Banci et al. 1995) but they should be more reliable since the only crystal structure available is for the bovine protein. The orientation of the rhombic perturbation, θ, is found to be +68.9°, with a standard error of 0.6° based on an assumed error of ±1 ppm for the ^{13}C shifts. This compares well with the in-plane rotation of the magnetic *y* axis,

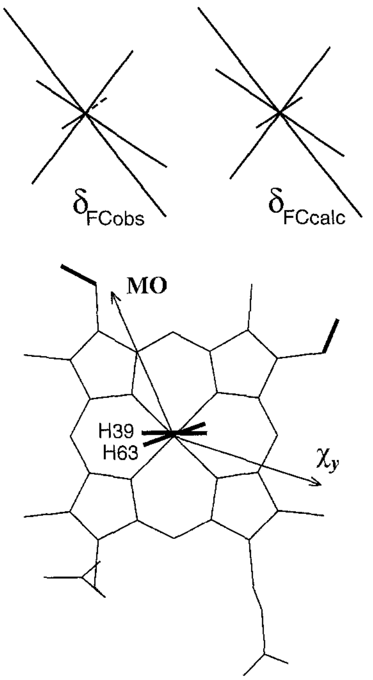

Fig. 1 Schematic representation of the observed (δ_{FCobs}) and calculated (δ_{FCcalc}) Fermi contact shifts of ^{13}C nuclei positioned α to the haem in the A form of bovine ferricytochrome b_5, and their relation to the orientation of the axial His ligands. The lengths of the vectors are proportional to the magnitudes of the Fermi contact shifts listed in Table 1 and they are directed towards the relevant substituents with the same orientation as the haem structure shown below. Note that the dashed line representing the observed shift of the 8^1CH carbon is based on a very weak signal at 110.5 ppm, which was not used in the analysis (see text). The vector labelled MO shows the orientation of the rhombic perturbation of the molecular orbitals, which is clearly close to the average of the normals to the two His ligands. The vector labelled χ_y shows the orientation of the magnetic y axis determined from ^1H dipolar shifts, which is rotated in the opposite sense to the rhombic perturbation with respect to the iron-nitrogen bonds

which is approximated by the sum of the Euler angles α and γ and found to be –63.1° (Banci et al. 1995), with an error of 1.2° based on an assumed error of ±0.02 ppm in the ^1H shifts. The orientation of the rhombic perturbation is also in reasonable agreement with the average of the orientations of the normals to the His imidazoles in the crystal structure (Mathews and Durley 1991), which is found at +54°. This comparison indicates that the two His ligands have approximately equal influence on the electronic structure and that together they dominate the rhombic perturbation, with the asymmetric haem substituents and the surrounding residues having only a minor effect. The similarity in the bonding of the two His ligands is also evident from the ^{13}C shifts of His 39 and His 63 βCH_2. The ^1H shifts of these groups have been assigned previously (McLachlan et al. 1988) and appear to be quite different because they are sensitive to the torsion angles of the side chain, but the difference was used to argue that one His is more strongly bound to the iron than the other (Lee et al. 1994). However, the ^{13}C shifts are clearly identifiable from cross peaks in Fig. 2 of Lee et al. (1995) and are close to each other at about 20 ppm. The ^{13}C shifts should be a more reliable indicator of electron delocalisation onto the imidazoles and hence of the His-Fe bonding, and it is clear that there is little difference between the two ligands.

Since the angle between the ring planes of the His ligands is small (19.3°), the splitting of the partially occupied π molecular orbitals ΔE should be close to a maximum (Walker et al. 1986; Turner et al. 1995). The value obtained, –4.02 kJ mol^{-1}, provides additional information about the strength of the interaction with the His N_3 π orbital and compares well with values in the region of –5 kJ mol^{-1} found for parallel His ligands in cytochrome c_3 (Turner et al. 1995), although cruder values for the molecular orbital coefficients were used in that study. The factor for the hyperfine coupling constant, –31.15±0.32 MHz is also within the expected range (Strom et al. 1972).

Finally, these calculations may be used to propose assignments for the ^{13}C resonances of the minor B form of bovine ferricytochrome b_5 and to test the structural model in which the A and B forms are assumed to differ simply by a 180° rotation of the haem about its 5CH-15CH axis (Keller and Wüthrich 1980; Mathews 1980). Since only two methyl resonances of the minor form can be assigned from the existing data, 7^1CH_3 at –61 ppm and 18^1CH_3 at –45 ppm, the analysis must be reduced to a single parameter. First we assume that the rhombic perturbation is determined entirely by the axial ligands and that they have the same orientation in relation to each other and to the haem plane in the two forms. The principal values of the magnetic susceptibility tensor should therefore be unchanged and the dipolar shift of the haem carbons may be calculated for any orientation given the modest assumption that the z axis coincides with the haem normal. We also assume that the hyperfine coupling constant is unchanged and so only the orbital mixing parameter, θ, is required, with the orientation of χ_y specified by $-\theta$. Optimising this single parameter gave a value of 8.3° and the shifts predicted for the B form, which are the simple sums of the dipolar shifts, contact shifts, and the diamagnetic references, are also listed in Table 1.

Since the Fermi contact shifts of the haem substituents are extremely sensitive to the orientation of the rhombic perturbation, which is determined primarily by the orientation of the axial ligands with respect to the haem, a change in electronic structure may be caused by a rotation of one or both ligands or by a rotation of the haem itself with respect to the rest of the protein. There is evidence for both types of modification: the resonances of Gly 42, which is hydrogen bonded to the ligand His 39, show significant shift differences between the two forms (Veitch et al. 1990), and the NOE between Val 61 and 12^1CH_3 in the A form has a different intensity to that between Val 61 and 18^1CH_3 in the B form (Pochapsky et al. 1990). The change in the shift of Gly 42 is difficult to interpret in the presence of a modified dipolar field, but the NOE data have been used to propose that the haem in the B form is rotated by a few degrees about the haem normal with respect to the A form, in addition to being flipped over about the 5CH-

15CH axis. The proposed sense of rotation shifts the 5CH of the B form towards the 20CH position found in the A form (Pochapsky et al. 1990), and Guiles et al. (1993) found a rotation of 5–10° between the magnetic susceptibility tensors in the A and B forms of rat cytochrome b_5 which they took to be a confirmation of the earlier conclusion. It is important to note that quite different reference frames apply: the NOE data relate the position of one methyl group in the protein to the position of the haem whereas the magnetic axes are determined with respect to the protein structure and do not provide any definitive information about the orientation of the haem itself. The analysis of Fermi contact shifts in the haem presented here yields the orientation of the rhombic perturbation with respect to the haem and the perturbation is related in turn to the magnetic axes fixed in the protein by theoretical considerations (Oosterhuis and Lang 1969). Despite the obvious limitations of the analysis of the B form of bovine ferricytochrome b_5, we find that the rhombic perturbation in the B form of bovine ferricytochrome b_5 is rotated with respect to that of the A form through a similar angle to that found in rat ferricytochrome b_5 (+12.8° compared with +3.5°) and that this is, within experimental error, equal to minus the rotation found for the magnetic axes. Thus, although the rotations of the rhombic perturbations and of the magnetic axes are apparently real, the studies of the oxidised proteins provide no evidence for any in-plane rotation of the haem between the two forms. The rotations, together with the increased splitting of the molecular orbitals found in the B form of rat ferricytochrome b_5 (Banci et al. 1995), could easily be explained by a rotation of His 39 in the B form to make its ring plane nearly parallel with the plane of His 63, which would retain the orientation found in the crystal structure.

Conclusion

The analysis of the newly available ^{13}C data for bovine ferricytochrome b_5 according to a model of π molecular orbitals with perturbed D_{4h} symmetry shows that the chemical shifts follow a simple pattern which provides a test of the assignments. The analysis also supports the view that the asymmetric distribution of unpaired electron density in the haem is dominated by the orientation of both of the axial ligands. However, the haem molecular orbital which is mainly responsible for the large paramagnetic shifts of substituents of pyrroles I and III noted by Lee et al. (1995) has an energy approximately 4 kJmol^{-1} higher than its orthogonal partner. The orbital of lower energy also has significant unpaired spin density and has its greatest amplitude on pyrroles II and IV. It is therefore certainly not obvious that facile electron transfer will occur via pyrrole III, as stated by Lee et al. (1995), and it would be necessary to consider the geometric and electronic structure of the electron transfer complex itself before reaching any such conclusion. Also, the chemical shifts calculated for the minor form of the bovine protein indicate that the electronic structure of the haem is closely similar to that of the minor form of the protein from rat. Comparison of the orientations of the rhombic perturbations with the principal axes of the magnetic susceptibility tensor clearly supports the view that the minor form is related to the major form by a 180° rotation about the 5CH-15CH axis, but neither the orientation of the magnetic axes nor the pattern of Fermi contact shifts confirm the proposal that there is an additional in-plane rotation of the haem. All of the observations could be explained, for example, by a rotation of just one of the His ligands through about 20°. However, we cannot conclude that there is zero in-plane rotation of the haem in the B form, but simply find that there are no data with sufficient accuracy to measure it.

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References

Banci L, Bertini I, Pierattelli R, Vila AJ (1994) ^{1}H-^{13}C HETCOR investigations on heme-containing systems. Inorg Chem 33: 4338–4343

Banci L, Pierattelli R, Turner DL (1995) Determination of haem electronic structure in cytochrome b_5 and metcyanomyoglobin. Eur J Biochem 232: 522–527

Bertini I, Luchinat C (1986) NMR of paramagnetic molecules in biological systems. Benjamin/Cummings, Menlo Park, CA

Byrn MP, Katz BA, Keder NL, Levan KR, Magurany CJ, Miller KM, Pritt JW, Strouse CE (1983) Low-spin ferric porphyrin complexes: analysis of the electronic structure based on single-crystal electron spin resonance measurements. J Am Chem Soc 105: 4916–4922

Griffith JS (1957) Theory of electron resonance in ferrihemoglobin azide. Nature 180: 30–31

Guiles RD, Altman J, Kuntz ID, Waskell L (1990) Structural studies of cytochrome b_5: complete sequence-specific resonance assignments for the trypsin-solubilized microsomal ferrocytochrome b_5 obtained from pig and calf. Biochemistry 29: 1276–1289

Guiles RD, Basus VJ, Sarma S, Malpure S, Fox KM, Kuntz ID, Waskell L (1993) Novel heteronuclear methods of assignment from a diamagnetic to a paramagnetic protein: application to rat cytochrome b_5. Biochemistry 32: 8329–8340

Harris G (1966) Low spin ferric hemoglobin complexes. Theoret Chim Acta 5: 379–397

Horrocks W, Greenberg ES (1973) Evaluation of dipolar nuclear magnetic resonance shifts in low-spin hemin systems: ferricytochrome *c* and metmyoglobin cyanide. Biochim Biophys Acta 322: 38–44

Karplus M, Fraenkel GK (1961) Theoretical interpretation of carbon-13 hyperfine interactions in electron spin resonance spectra. J Chem Phys 35: 1312–1323

Keller RM, Wüthrich K (1972) The electronic g-tensor in cytochrome b_5: high resolution proton magnetic resonance studies. Biochim Biophys Acta 285: 326–336

Keller RM, Wüthrich K (1980) Structural study of the heme crevice in cytochrome b_5 based on individual assignments of the ^{1}H-NMR lines of the heme group and selected amino acid residues. Biochim Biophys Acta 621: 204–217

Kurland RJ, McGravey BR (1970) Isotropic NMR shifts in transition metal complexes: the calculation of the Fermi contact and pseudocontact terms. J Magn Reson 2: 286–301

La Mar GN, Viscio DB, Smith KM, Caughey WS, Smith ML (1978) NMR studies of low-spin ferric complexes of natural porphyrine derivatives. 1. Effect of peripheral substituents on the π electronic asymmetry in biscyano complexes. J Am Chem Soc 100: 8085–8092

La Mar GN, Walker FA (1979) NMR of paramagnetic porphyrins. In: Dolphin D (ed) The porphyrins, vol 4. Academic Press, New York, pp 61–157

Longuet-Higgins HC, Rector CW, Platt JR (1950) Molecular orbital calculations on porphine and tetrahydroporphine. J Chem Phys 18: 1174–1181

Lee KB, La Mar GN, Mansfield KE, Smith KM, Pochapsky TC, Sligar SG (1993) Interpretation of hyperfine shift patterns in ferricytochromes b_5 in terms of angular position of the heme: a sensitive probe for peripheral heme protein interactions. Biochim Biophys Acta 1202: 189–199

Lee KB, McLachlan SJ, La Mar GN (1994) Hydrogen isotope effects on the proton nuclear magnetic resonance spectrum of bovine ferricytochrome b_5: axial hydrogen bonding involving the axial His-39 imidazole ligand. Biochim Biophys Acta 1208: 22–30

Lee KB, Kweon J, Park H (1995) Assignment of hyperfine-shifted heme carbon resonances in ferricytochrome b_5. FEBS Lett 367: 77–80

Mathews FS (1980) The orientation of the heme group in crystalline cytochrome b_5. Biochim Biophys Acta 622: 375–379

Mathews FS, Durley RCE (1991) entry 3B5C. Brookhaven Protein Data Bank

McLachlan SJ, La Mar GN, Lee KB (1988) One- and two-dimensional nuclear Overhauser effect studies of the electronic/molecular structure of the heme cavity of ferricytochrome b_5. Biochim Biophys Acta 957: 430–445

Oosterhuis WT, Lang G (1969) Mössbauer effect in $K_3Fe(CN)_6$. Phys Rev 178: 439–456

Pierattelli R, Banci L, Turner DL (1996) Indirect determination of magnetic susceptibility tensors in peroxidases: a novel approach to structure elucidation by NMR. J Biol Inorg Chem (in press)

Pochapsky SJ, La Mar GN (1990) Relationship between heme binding-site structure and heme orientation of two ferrocytochrome b_5s. A study in prosthetic group recognition. J Am Chem Soc 112: 5258–5263

Shulman RG, Glarum SH, Karplus M (1971) Electronic structure of cyanide complexes of hemes and heme proteins. J Mol Biol 57: 93–115

Strom ET, Underwood GR, Jurkowitz D (1972) On the linear dependence of ^{13}C spin polarization parameters. Mol Phys 24: 901–904

Timkovich R (1991) Heteronuclear multiple-quantum coherence NMR spectroscopy of paramagnetic heme and cytochrome c_{551}. Inorg Chem 30: 37–42

Traylor TG, Berzinis AP (1980) Hemoprotein models: NMR of imidazole chelated protohemin cyanide complexes. J Am Chem Soc 102: 2844–2846

Turner DL (1995) Determination of haem electronic structure in His-Met cytochromes *c* by ^{13}C-NMR. The effect of the axial ligands. Eur J Biochem 227: 829–837

Turner DL, Salgueiro CA, Schenkels P, Le Gall J, Xavier AV (1995) Carbon-13 NMR studies of the influence of axial ligand orientation on haem electronic structure. Biochim Biophys Acta 1246: 24–28

Veitch NC, Whitford D, Williams RJP (1990) An analysis of pseudocontact shifts and their relationship to structural features of the redox states of cytochrome b_5. FEBS Lett 269: 297–304

Walker FA, Huynh BH, Scheidt WR, Osvath SR (1986) Models of cytochrome *b*. Effect of axial ligand plane orientation on the EPR and Mössbauer spectra of low-spin ferrihemes. J Am Chem Soc 108: 5288–5297

Wüthrich K, Baumann R (1973) Hyperfine shifts of the ^{13}C-NMR in low spin iron(III) porphyrin complexes. Helv Chim Acta 56: 585–596