Structural Differences of the Iron–Dioxygen Moiety of Haemoprotein Models with and without an Axial Hindered Base as Revealed by ^{17}O NMR and FTIR Spectroscopy in Solution

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The N–H stretching vibrations of the oxygenated 'hybrid' haemoprotein models with an axial hindered base indicate that there is no conventional hydrogen bond with the terminal oxygen of the $Fe–O_2$ moiety, contrary to the models with an axial unhindered base; however, the $Fe–O_2$ moiety is highly polarizable as indicated by ^{17}O NMR spectroscopy.

The steric bulk of certain axial ligands (*e.g.* 1,2-dimethylimidazole) of iron(II) haemoprotein synthetic models, hinders the approach of the iron atom into the plane of the porphyrin[1–4] and provides models of the lowered O_2 affinity and the T (tensed)-state of haemoproteins.[5–9] Numerous investigations of these model systems with a variety of physical and structural probes demonstrate that the effect of the 2-Meim† ligand compared to the 1-Meim† analogue is mainly in lengthening of the Fe–O bond, thus providing a direct connection between bond length and O_2 affinity.[3,8,10–12] In this communication, we introduce for the first time ^{17}O NMR and Fourier transform infrared (FTIR) spectroscopies as tools of investigating electronic differences of the $Fe–O_2$ moiety of haemoprotein models with and without an axial hindered base.[13]† It is demonstrated that in the models with an axial hindered base, in addition to lengthened Fe–O bond, there is no conventional hydrogen bond owing to the unfavourable position of the terminal oxygen relative to the NH of the distal moieties, though, the dioxygen moiety is highly polarized due to dipolar interactions at distances longer than conventional hydrogen bonds.

The O_2 off rates, $k^-_{O_2}$, are generally markedly higher in the models with an axial hindered base than those with an unhindered axial base, therefore, a judicious choice of the appropriate compounds with relatively slow O_2 off rates was necessary in order to avoid extensive broadening of the [17]O resonances. The so-called 'hybrid' haemoprotein models which have two pivalamido pickets and an amide handle of variable length linked in a cross-*trans* configuration[13] could be considered as appropriate candidates since $k^-_{O_2}$ are relatively small.[14]‡

Fig. 1 shows the ^{17}O NMR spectra of the 'hybrid' compounds' 1a and b.§ The spectra exhibit two distinct resonances in agreement with the bent end-on geometry first proposed by

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† *Abbreviations used*: TivPP, *meso*-tetra($\alpha,\alpha,\alpha,\alpha$-*o*-pivalamidophenyl)porphyrinato; 1-Meim, 1-methylimidazole; 1,2-Me$_2$im, 1,2-dimethylimidazole; amide·BHP, basket handle porphyrin in amide series (M. Momenteau, J. Mispelter, B. Loock and J.-M. Lhoste, *J. Chem. Soc., Perkin Trans. 1*, 1985, 221); ether·BHP, basket handle porphyrin in ether series (M. Momenteau, J. Mispelter, B. Loock and J.-M. Lhoste, *J. Chem. Soc., Perkin Trans. 1*, 1985, 61).

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‡ Kinetic rate constants of 1a and b for the binding of O_2 are:

	$k^-_{O_2}$ 10^{-3}/s^{-1}	k_{O_2} 10^{-7}/dm^3 mol^{-1} s^{-1}	
1a	0.002	0.22	(Ref. 15)
1b	0.0147	0.054	(unpublished results)

Further, the ratio of oxygen affinities of 1a and b is ~38:1, rather similar to the ratio of 100:1 observed for R and T haemoproteins.[4]

§ 1a and b were synthesized following literature procedures (see ref. 14). Gaseous oxygen containing ~38% [17]O (CEA, France) was added to the organic solutions and the tube (10 mm) was sealed under an O_2 pressure of 2–3 atm.
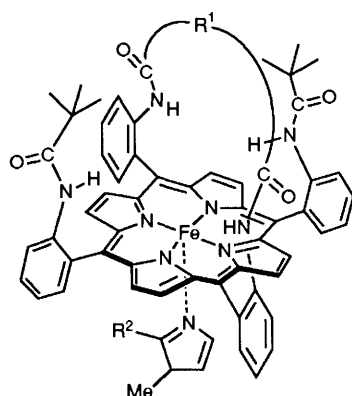
1a R^1 = -[CH$_2$]$_6$-, R^2 = H
**1b** R^1 = -[CH$_2$]$_6$-, R^2 = Me

Pauling[17] for haemoglobin and revealed by the X-ray crystal structures of oxygenated Fe^{II} picket fence porphyrin[3] and oxyhaemoglobin.[18] The resonance of the oxygen atom coordinated to iron¶ is shifted by 30–39 ppm and that of the terminal oxygen by 50–60 ppm to a low frequency in model **1b** relative to that in model **1a**. It has been established that in a variety of transition-metal compounds there is a poor but real correlation between ^{17}O chemical shifts and metal–oxygen π-bond character.[19] Similar correlation was also suggested for haem models with an unhindered axial base.[16] Therefore, the low frequency shift of the resonance of the oxygen coordinated to iron in model **1b** could reflect an increase in the Fe–O length (decrease in the Fe–O π-bond order‖). This point is firmly established from X-ray structural studies of the picket fence porphyrin models in the ligated and unligated state.[3] The bulky 2-methyl substituent on the 2-Meim ligand results in lengthened axial bonds relative to the sterically less demanding 1-Meim ligand.[20] The sum of the Fe–O and Fe–N$_{im}$ bond lengths is 4.005 Å for 2-Meim, but only 3.813 Å for Fe(O$_2$) (TpivPP) (1-Meim).† Of this 0.192 Å difference, most (0.15 Å) arises from a lengthening of the Fe–O bond.

The shift of the terminal oxygen atom to low frequency in the model **1b** relative to that in **1a** is in the same direction and larger in magnitude to those observed in 'hybrid' or 'basket handle' porphyrins (amide·BHP) bearing NH groups in the handles relative to the ether·BHP in which no hydrogen bonding interactions are expected.[16] This significant low frequency shift appears surprising and demonstrates that the π effect which weakens the Fe–O$_2$ bond but strengthens the O–O bond is not operative in this particular case.[21] We suggest that this low frequency shift is due to a significant polarization of the O–O bond. In terms of the spin-pairing model[22] the iron–oxygen bond is a double bond with the π-bond polarized as Fe$^-$–O$^+$=O. A weakening of the iron–oxygen bond will weaken the π-bond more than the σ-bond and make the bound O$_2$ more negative. The N–H stretching vibrations, however, indicate the absence of conventional hydrogen bonding interactions in the 'hybrid' porphyrin models with an axial hindered base, Fig. 2, contrary to the 'hybrid' models with an
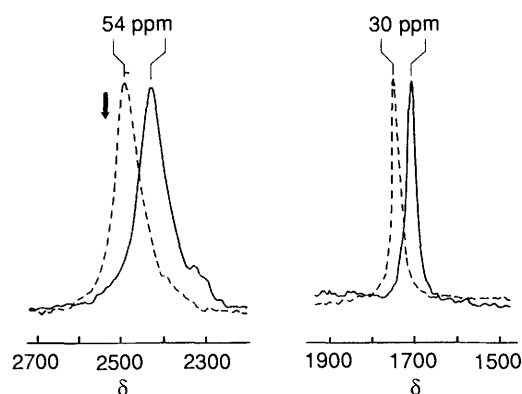
Fig. 1 ^{17}O NMR spectra (54.2 MHz) of the oxygenated compounds **1b** (———) and **1a** (- - - -) from use of the normal 90° pulse with a preacquisition delay time Δt ~ 50 μs in order to eliminate acoustic ringing problems[15,16] and recorded in two steps with the carrier frequency on the absorption resonances. Concentration ≅ 10^{-2} mol dm^{-3} in toluene solution. [1,2-Me$_2$im] ≅ 5 × 10^{-1} mol dm^{-3}, T = 297 K, number of scans 1 080 000 (line-broadening filtering LB = 1 kHz) and 350 000 (LB = 500 Hz) for the high- and low-frequency resonances, respectively. Chemical shifts were measured relative to 1,4 dioxane (+0.2 ppm relative to H$_2$O at 303 K) determined in a separate replacement technique.[16]

The ↓ denotes the region of the resonance position of the terminal oxygen of the picket-fence porphyrin model, with an excess of 1-Meim, and ether·BHP in which no hydrogen bonding interactions are expected.[3,4,13]
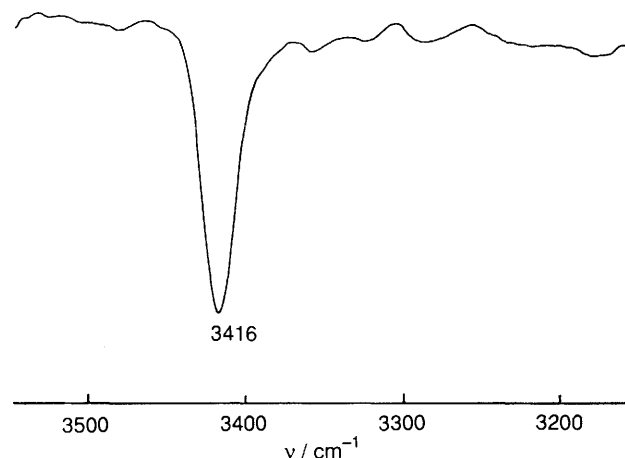
Fig. 2 FTIR spectrum of the oxygenated compound **1b** of the N–H amide stretching region in toluene solution

axial unhindered base which exhibit an absorption band at 3370–3350 cm^{-1} due to the hydrogen bonded N–H group.[14] The high polarizability of the dioxygen moiety in the models with an axial hindered base can be interpreted by assuming dipolar interactions at distances longer than conventional hydrogen bonds.** Our results therefore present compelling experimental evidence in support of recent approximate CFF calculations of Jameson and Drago.[23] These authors suggested that even with small amounts of charge transfer into the O$_2$

¶ Assignment of the resonances has been achieved on the basis of extended Hückel calculations and compared to the ^{17}O resonances of ozone.[15]

‖ Similarly, resonance Raman studies of picket-fence model dioxygen complexes in the solid state have shown a decrease of ν$_{Fe-O}$ of the model (1,2-Me$_2$im)Fe(TpivPP)(O$_2$) (ν$_{Fe-O}$ ≅ 561–558 cm^{-1}, depending on temperature) relative to that for (1-Meim)Fe(TPivPP)(O$_2$) (ν$_{Fe-O}$ ≅ 570–567 cm^{-1}). These results are also consistent with a longer Fe–O bond in the 1,2-Me$_2$im complex, although it should be emphasized that the steric influence of the 2-methyl group on ν$_{Fe-O}$ seems to be relaxed somewhat in solution.[10,11]

** The remarkable sensitivity of the Fe–O$_2$ moiety to long range dipole–dipole interactions has already been noticed by Chang and Traylor (C. K. Chang and T. G. Traylor, *Proc. Natl. Acad. Sci. USA*, 1975, **72**, 1166). These authors observed that oxygenation of several model haem compounds is enhanced by aprotic polar solvents such as dimethylacetamide. The increased oxygen affinity with increased solvent polarity was found to be almost entirely due to the off rates. Preliminary ^{17}O NMR spectroscopy results indicate significant and systematic changes of the ^{17}O chemical shifts of the FeO$_2$ moiety of the picket fence model (1-Meim)Fe(TpivPP)(O$_2$) in CH$_2$Cl$_2$ upon increasing the amount of dimethylformamide, although, no classical hydrogen bond is expected with the added cosolvent.

ligand, a substantial attraction exists at distances O···H–N ~ 4 Å, *i.e.* much longer than conventionally taken as indicative of hydrogen bonds and despite the non-linear arrangement of the N–H···O atoms.

Our results are important for several reasons. They represent the first direct evidence that in the 'hybrid' models with an axial hindered base the dioxygen moiety does not form a conventional hydrogen bond with the N–H of the distal handles contrary to the models with an axial unhindered base. Further, it is demonstrated that the 1,2-Me$_2$im ligand results not only in lengthening of the Fe–O$_2$ bond, as it is widely adopted in the literature, but in a strong polarization of the O–O bond. Lengthening of the Fe–O bond results in higher dissociation rates and the strong polarization of the dioxygen bond in higher autoxidation rates in excellent agreement with experimental data.[14]

It should be emphasized that recent X-ray structural studies in oxyhaemoglobins revealed almost identical stereochemical features of the O–O bond in the T (tensed) and R (relaxed) state[7–9] while in the case of 'picket fence' porphyrin oxygenated derivatives only a lower band for the O–O separation (1.21 Å) and an upper band for the Fe–O–O angle (129°) was defined due to statistical disorder.[3] Our results, therefore, clearly demonstrate that ^{17}O NMR and FTIR spectroscopy might become a unique structural probe in studying structural differences of the Fe–O$_2$ moiety of haemoprotein models with and without an axial hindered base.

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