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Hydrogen-Bond Stabilization of Dioxygen, Conformation Excitation, and Autoxidation Mechanism in Hemoprotein Models As Revealed by ^{17}O NMR Spectroscopy

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Abstract: Oxygen-17 NMR spectra of the Fe–O_2 moiety of five closely related dioxygen hemoprotein models have been recorded in organic solvents. The spectra exhibit two well-resolved resonances in agreement with the end-on structure proposed by Pauling for oxyhemoglobin. The sensitivity of oxygen-17 chemical shifts to hydrogen-bonding interactions with distal moieties is demonstrated for the first time. The unusual temperature dependence of the oxygen-17 line widths of (O_2)FeTpivPP(1-MeIm) can be attributed to a dynamic equilibrium of two conformers of the Fe–O–O unit with a substantially different electric field gradient tensor at both oxygen sites and a small difference in energy which is dominated by O_2···pivalamido interactions. Detection of $H_2{}^{17}O_2$ and $H_2{}^{17}O$ species strongly supports the implication of the superoxide ion (or the hydroperoxyl radical $HO_2^\bullet$) in the autoxidation reaction.

The binding of dioxygen to hemoproteins and synthetic model compounds has long been a topic of interest, extensive research activity, and debate.[1-4] The bent structure of the dioxygen molecule, first proposed by Pauling,[5] was found in oxygenated hemoproteins[6-8] and in oxygenated iron(II) porphyrins both in solid state[9-11] and in solution.[12-15] Additionally, recent neutron diffraction studies of oxymyoglobin[7] and X-ray structure determination of oxyhemoglobin[8] have provided strong direct evidence that the dioxygen ligand forms a hydrogen bond with the distal histidine residue (His E7). In synthetic models, the so-called basket-handle porphyrins[4] (BHP), the presence of secondary amide groups near the binding site of the O_2 molecule also induces an intramolecular hydrogen bonding interaction between the amide proton and the terminal oxygen atom.[12,13] This specific interaction has a strong effect on O_2 affinity and decreases the dissociation rate constant, hence contributing to the stabilization of the dioxygen adduct.[12,14] In the picket-fence porphyrin model, (O_2)-FeTpivPP(1-MeIm), and in HbO_2 a rotational model of the oxygen molecule was proposed by Spartalian et al.[16] to explain the temperature dependence of the Mössbauer quadrupole splitting, although the temperature dependence of the line widths was not adequately explained.[17] Tsai et al.[18] suggested that the temperature dependence of the Mössbauer quadrupole splitting of HbO_2, which is assumed to be in a quantum mixture of ferrous, Fe(d^6)O_2, and ferric, Fe(d^5)O_2^-, states of the Mulliken's electron donor–acceptor type, could be explained by anharmonic vibration of the oxygen molecule in the iron ion's binding well and by an increase of the ferric state fraction. Others suggested[19] that the quadrupolar splitting in the Mössbauer spectra could be explained in terms of a totally paired Fe(d^6)O_2 configuration.

In view of the great importance of distal and proximal effects in the nature of the metal–oxygen bond and the control of dioxygen affinity,[4,20] it is clear that a new methodology that could probe directly the electronic changes of the Fe–O_2 moiety would prove very useful. ^{17}O NMR spectroscopy can be considered as an obvious candidate; however, it was only recently that the first high-resolution ^{17}O NMR spectrum of a single-face hindered iron porphyrin–dioxygen complex in toluene solution was reported.[21] In this paper, we report the ^{17}O NMR spectra of the Fe–O_2 moiety of five closely related model compounds, Chart I, of the active site of oxygen carrier hemoproteins in solution. The sensitivity of oxygen-17 chemical shifts and nuclear quadrupole coupling constants to hydrogen bonding and long-range dipole–dipole interactions with the distal environment is demonstrated for the first time. Additionally, the detection of $H_2{}^{17}O$ and $H_2{}^{17}O_2$ species provides some evidence for the mechanism of the autoxidation process of iron(II) hemoprotein model compounds.

Experimental Section

Chemicals. Chloroiron(III) basket-handle porphyrins,[22,23] chloroiron(III) picket-fence porphyrin,[24] and chloroiron(III) hybrid porphyrin[15]

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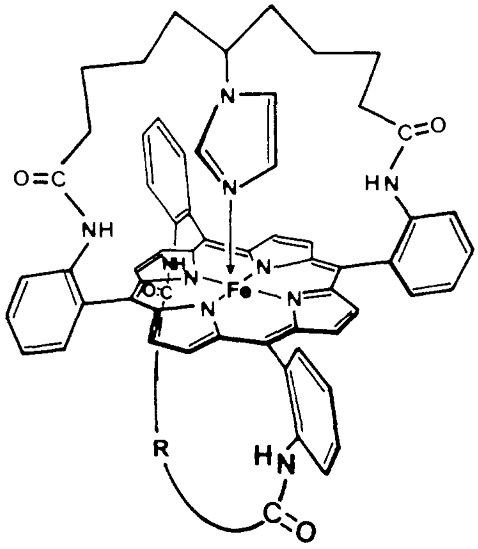
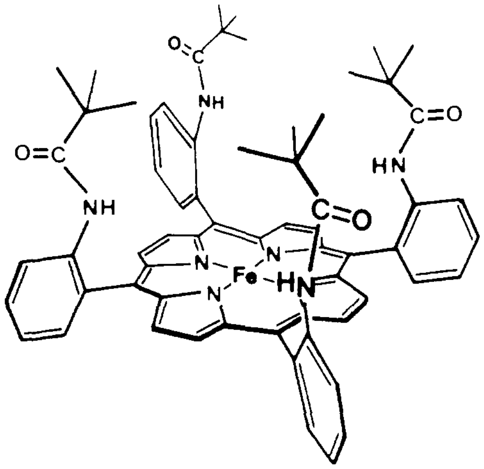
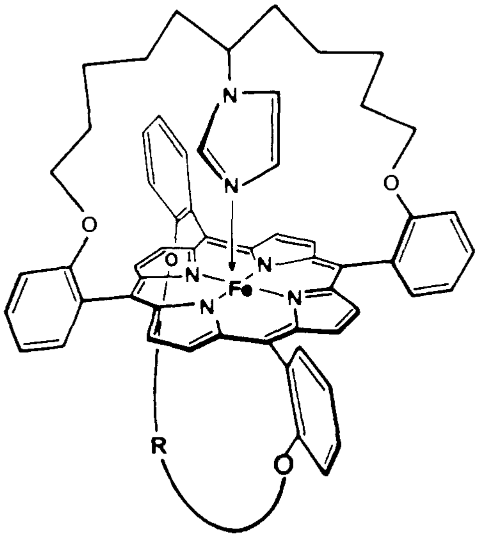
Chart I



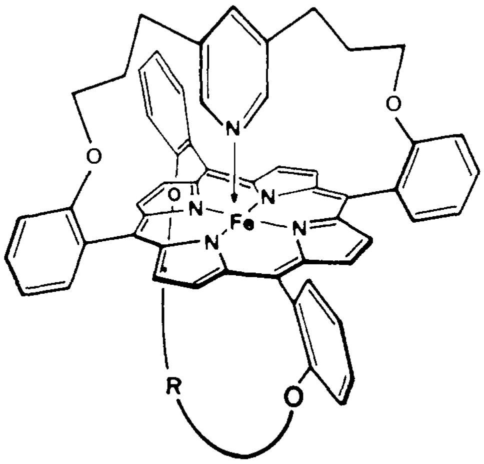
1 R= $-(CH_2)_{10}-$



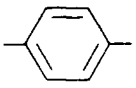
2



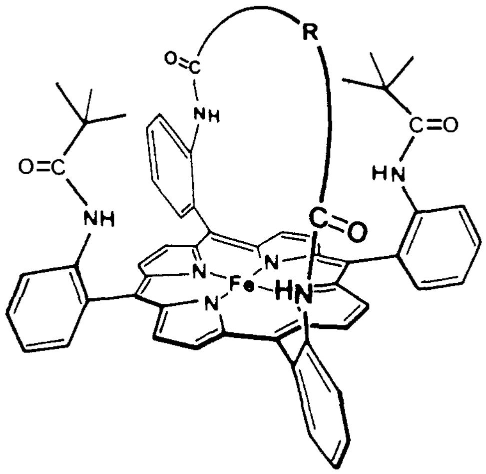
3 R= $-(CH_2)_{12}-$



4 R= $-(CH_2)_3-$



$-(CH_2)_3-$



5 R= $-(CH_2)_6-$

6 R= $-(CH_2)_8-$

were synthesized following literature procedures. Fe(II) complexes were obtained by reduction of Fe(III) species in methylene chloride or in toluene with an aqueous sodium dithionite solution under argon.[25] The reduced compounds ($\sim 10^{-2}$ M) in the organic phase were then transferred under argon into the NMR tube via a stainless steel tube. 1-Methylimidazole (Aldrich) was distilled under reduced pressure before use and was added (10^{-1} M) to the reduced solutions of single-face hindered porphyrins. Gaseous oxygen containing 50% oxygen-17 (Yeda-Stable Isotopes, Rehovot, Israel) was added to the organic solutions, and the tube was sealed under an O_2 pressure of a 1.5–3 atm. For the picket-fence porphyrin, additional experiments were performed with gaseous oxygen containing 70% and 33% oxygen-17.

^{17}O NMR Measurements. The ^{17}O NMR data were obtained with a Bruker AM-400 multinuclear spectrometer operating at 54.2 MHz for ^{17}O. The field was optimized with D_2O, and no locking system was used during accumulation. The temperature was adjusted with the use of a calibrated thermoresistance introduced into the magnet by way of a cylindrical tube containing the same solvent. Chemical shifts were measured relative to 1,4-dioxane (+0.2 ppm relative to H_2O at 303 K) determined in a separate replacement experiment. Data manipulations were carried out on an Aspect-3000 computer. The following spectral parameters were used: spectral width = 100 kHz; 90° pulse length at the carrier frequency = 30 μs; quadrature phase detection; acquisition time = 5 ms; zero-filling to 2 K before Fourier transform. Line widths were estimated by simulation of the ^{17}O resonances with a Lorentzian line-shape function (Bruker DISNMR program).

To eliminate acoustic ringing problems, the spectra were recorded either with a preacquisition delay time of 50–100 μs for line widths < 2 kHz or with the RIDE version of the three-pulse sequence[26] for line widths > 2 kHz. Because of the limited power of the 90° and 180° pulses it was necessary to record the spectra in two steps with the carrier frequency on each absorption resonance. ^{17}O longitudinal relaxation times were measured by the inversion–recovery method using 16 values of τ in the pulse sequence $(180°{-}\tau{-}90°{-}T_{acq})_n$. T_{acq} was $>6T_1$ and n = 50 000. The T_1 values were determined by a three-parameter nonlinear least-squares fit.

Results and Discussion

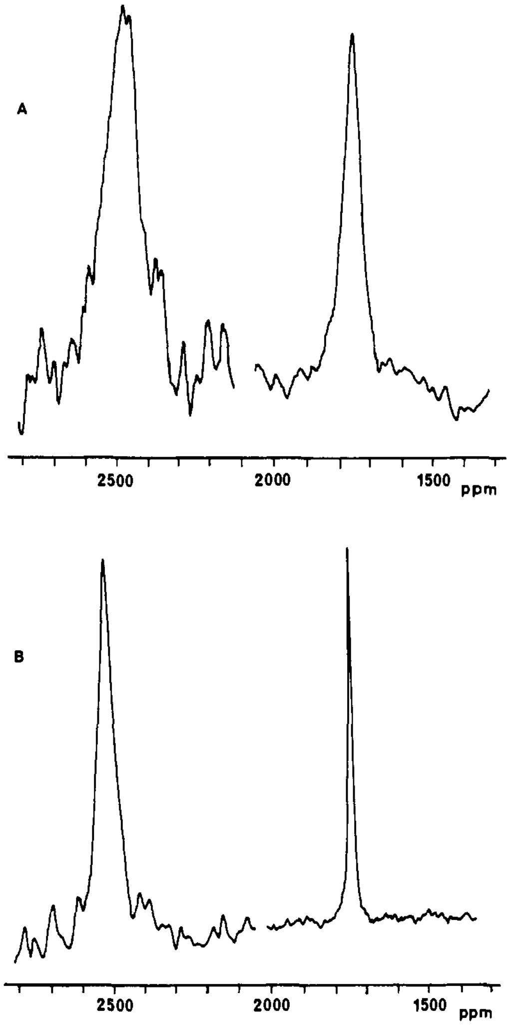
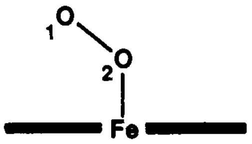
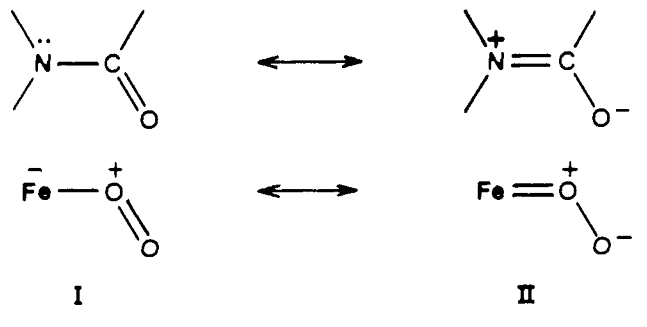


Figure 1. (A) ^{17}O NMR spectrum of compound **1** from use of the RIDE version of the three-pulse sequence[26] and recorded in two steps with the carrier frequency on the absorption resonances: concentration $\sim 1.5 \times 10^{-2}$ M in CH_2Cl_2, T = 283 K, SW = 100 kHz, line-broadening filtering LB = 1 kHz, number of scans = 400 000. The pulse and preacquisition delays were set to minimum and the time domain data block was shifted by three complex pairs of points (Δt = 15 μs). (B) ^{17}O NMR spectrum of compound **2** from use of the normal 90° pulse sequence and recorded in two steps with the carrier frequency on the absorption resonances: concentration 1.5×10^{-2} M in CH_2Cl_2, concentration of 1-MeIm 2.5×10^{-1} M, T = 273 K, SW = 71.5 kHz, preacquisition delay time Δt = 50 μs, number of scans $\sim$ 600 000 and $\sim$130 000 for the high- and low-frequency resonances, respectively; line-broadening filtering LB = 500 Hz.



Scheme I



Chemical Shifts: Effects of Hydrogen Bonding and Long-Range Dipole–Dipole Interactions[27] with Distal Moieties. Figure 1 shows the ^{17}O NMR spectra of compounds **1** and **2** in methylene chloride at 283 K. Both spectra exhibit two distinct resonances in agreement with the end-on angular bond first proposed by Pauling[5] for hemoglobin and revealed by the X-ray crystal structures of oxygenated iron(II) picket-fence porphyrin **2**[9–11] and oxyhemoglobin.[7] The chemical shift of the terminal oxygen atom, O(1), of compound **1** is very close to that observed in $Fe(O_2)$-aP$(Piv)_2(C_{10})$, **6**,[21] in toluene while the resonance of the oxygen atom coordinated to the iron ion, O(2), is slightly shifted to higher frequency. This suggests that the electronic environment of the $Fe{-}O_2$ moiety, and therefore hydrogen bonding with the distal amide proton, is similar in both compounds. With the picket-fence porphyrin model **2** in the presence of an excess of 1-methylimidazole as proximal ligand, a shift of ca. 31 ppm of the resonance of the terminal oxygen atom to higher frequency is observed at 283 K while the resonance of the oxygen atom coordinated to iron is shifted to lower frequency by $\sim$15 ppm. This observation demonstrates the absence of hydrogen bonding between the terminal oxygen and the NH amides of the pickets in the Collman's model, confirming the IR studies,[15] while the orientations of oxygen molecule in the crystal indicate weak dipole–dipole interactions (see discussion of the line widths). A shift to higher frequency resulting from a decrease in hydrogen bonding has been reported in a variety of oxygen functional groups.[28] More specifically it has been suggested that

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(27) Hydrogen bonding can be placed in a broader category of weak interactions where dipole (electric) effects happen to be an essential ingredient. However, it is usually adopted in the literature that at distances longer than the van der Waals radii of the donor and acceptor atoms the notion that there is a hydrogen bond must be discarded, although long-range dipole–dipole interactions might occur.

Table I. ^{17}O Chemical Shifts,[a] Line Widths,[b] Chemical Shift Differences $\Delta\delta$, Ratios of the Line Widths, and the Expressions $\chi^2(1 + \eta^2/3)$ of the Oxygen of the $Fe-O_2$ Moiety of the Hemoprotein Models of Chart I

compd	solvt	concn,[c] × 10^2 M	temp, K	δ_2,[d] ppm	$(\Delta\delta_{1/2})_2$,[d] Hz	δ_1,[e] ppm	$(\Delta\nu_{1/2})_1$,[e] Hz	$\Delta\delta$, ppm	$(\Delta\nu_{1/2})_1/(\Delta\nu_{1/2})_2$	$\chi^2(1 + \eta^2/3)_1/\chi^2(1 + \eta^2/3)_2$
1	CH_2Cl_2	1.5	273	1764.8	2640	*f*	*f*			
1	CH_2Cl_2	1.5	283	1762.5	2280	2486.9	6450	724.4	2.83	1.68
2	CH_2Cl_2	2.5	253	1750.0	410	2524.0	3340	774.0	8.15	2.85
2	CH_2Cl_2	2.5	263	1749.0	380	2521.8	2910	772.8	7.66	2.77
2	CH_2Cl_2	2.5	273	1747.9	370	2518.7	2480	770.8	6.70	2.59
2	CH_2Cl_2	2.5	283	1747.3	450	2518.2	1960	770.9	4.36	2.09
2	CH_2Cl_2	2.5	298	1744.9	1090	2512.6	2070	767.7	1.90	1.37
2	CH_2Cl_2	2.5	303	1747.0	1680	2512.2	2770	765.2	1.65	1.28
3	CH_2Cl_2	1.8	273	1754.5	990	2514.6	3750	760.1	3.79	1.95[g]
4	toluene	1.0	263	*h*	*h*	*h*	*h*			
5	toluene	1.4	253	1737.8	1770	*f*	*f*			
5	toluene	1.4	273	1738.0	910	2480.5	3390	743.7	3.73	1.93
5	toluene	1.4	283	1739.4	710	2479.9	3170	740.5	4.46	2.11
5	toluene	1.4	288	1739.5	660	2480.1	3100	740.6	4.70	2.17
5	toluene	1.4	297	1739.5	600	2484.2	2480	744.7	4.13	2.03
5	toluene	1.4	307	1740.2	560	2485.7	2040	745.5	3.64	1.91
6[i]	toluene	2.5	273	1760.5	2017	*f*	*f*			
6[i]	toluene	2.5	283	1757.9	1399	2484.7	4319	726.8	3.08	1.75
6[i]	toluene	2.5	297	1756.3	890	2487.7	3220	731.4	3.61	1.90
6[i]	toluene	2.5	307	1755.0	811	2488.3	2584	733.3	3.18	1.78

[a] Chemical shifts are reported relative to external 1,4-dioxane by using the sample replacement technique. Estimated errors from ±0.6 to ±2.5 ppm, depending on the line width of the resonance. Positive values indicate deshielding. [b] Line widths of the resonances at half-height corrected for the line-broadening factors. Estimated error <5% for line widths up to 2 kHz, ~10% for line widths larger than 2 kHz. [c] Upper values since some precipitation of the compounds was observed during experimentation. [d] Oxygen coordinated to iron. [e] Terminal oxygen. [f] The breadth of the resonance prohibits accurate estimation of spectral parameters. [g] Reference 46. [h] No signal. [i] Reference 21.

in amides and peptides the elimination of a hydrogen bond acting on the oxygen lone pair results in a chemical shift to higher frequency of 18–22 ppm.[29-32] The precise value of this shift depends on the strength and geometry of the particular hydrogen bonding. This tendency can be interpreted qualitatively by assuming that reduction in hydrogen bonding enhances the resonance structure I (Scheme I).[29,30] A similar scheme can be proposed to explain the low-frequency shift in the $Fe-O_2$ moiety. It has been recently 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[33] as for the carbon–oxygen and nitrogen–oxygen bonds.[28] It is expected that an increase in hydrogen bonding of the $Fe-O_2$ moiety enhances the resonance structure II (Scheme I) by increasing the metal–oxygen π-bond order (high-frequency shift) and decreasing the O–O π-bond order (low-frequency shift), in agreement with the experimental chemical shift data. Though there appear to be some generalities in the relations of ^{17}O chemical shifts and π-bond orders, caution is necessary for their interpretation because of the limited number of definite results available and the possibility of change in the Fe–O–O bond angle (see discussion of the line widths). Recent theoretical calculations of the ^{17}O NMR chemical shifts of ozone[34] (which can be considered as a prototype molecule of the electronic structure of the $Fe-O_2$ moiety[21]) have shown that they are strongly dependent on small changes in bond angle and bond length.

The remarkable stability and good solubility of the picket-fence porphyrin oxygen adduct **2**, in the presence of an excess of **1**, methylimidazole, allowed us to study this species in a large range of temperatures (Table I). On increase of the temperature a small but systematic variation of the chemical shifts of both oxygen atoms to lower frequencies, larger for the terminal oxygen, was observed. This shift is presumably due to an equilibrium of two conformers with different M–O–O angles (see discussion of the line widths).

Similar experiments were carried out with compounds **3**–**5** (Chart I). Compound **3**, in which the superstructures are linked to the macrocycle by ether groups, exhibits chemical shifts similar to those of the picket-fence porphyrin **2**, confirming the absence of hydrogen bonding. Several attempts to observe the ^{17}O NMR resonances of compound **4** were unsuccessful. This is probably due to a high dissociation rate constant,[4] which results in an extensive broadening of the resonances.

Compound **5**, which exhibits a good solubility and a high stability in toluene solution, was studied over a wide range of temperatures. The chemical shifts of the terminal oxygen atom (Table I) confirm the presence of hydrogen bonding between the oxygen ligand and the amide protons of the handle. However, the resonance of the oxygen atom coordinated to iron is shifted by 15–20 ppm to low frequency relative to those observed for compounds **1** and **6**.[21] Such a particular chemical shift could reflect an increase in the Fe–O bond length (decrease in the Fe–O π-bond order) and/or a difference in the Fe position relative to the average plane of the porphyrin ring. This latter point is firmly established from a recent structural determination by X-ray diffraction of the carbonyl derivative of compound **5**.[35] In this compound the macrocycle and the superstructure are very distorted, whereas in several analogues bearing a longer handle no distortion was observed.

Oxygen-17 NMR Line Widths: Evidence for Conformational Excitation. The predominant relaxation mechanism for ^{17}O is quadrupolar in nature.[28] The resonance line width, therefore, is directly related to the square of the nuclear quadrupole coupling constant, $\chi = e^2q_{zz}Q/h$, and the effective rotational correlation time, τ_c, at the site of the ^{17}O nucleus:

$$\Delta\nu_{1/2} = \frac{1}{\pi T_2} = \frac{12\pi^2}{125}\left(1 + \frac{\eta^2}{3}\right)\chi^2\tau_c \qquad (1)$$

where eq_{zz} is the largest component of the field gradient tensor at the nucleus due to the electrons, Q is the quadrupole moment of the nucleus (-2.63×10^{-30} m^2), and η is the asymmetry pa-

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rameter[36] (the motional narrowing limit $\omega_0^2\tau_c^2 \ll 1$ is assumed, where ω_0 is the nucleus Larmor frequency). A decrease in the line widths is expected on increasing the temperature due to an increase in the correlation time for molecular tumbling at the site of the superoxo moiety. Examination of the line-width data in Table I reveals, first, that the line widths of the oxygen bound to iron are significantly smaller than those of the terminal oxygen nucleus. Since the rotational correlation time of both oxygens is expected to be identical, it can be concluded that the expression $\chi^2(1 + \eta^2/3)$ and therefore the electric field gradient tensor are significantly different at both oxygen nuclei. Second, the line-width variation as a function of temperature of the compounds **5** and **6** is in the direction predicted by eq 1. In the picket-fence porphyrin **2**, however, the line width of the O(2) resonance exhibits a minimum at 273 K and then increase dramatically upon increasing the temperature. The line width of the O(1) resonance exhibits a minimum around 283 K and increases at higher temperatures. Several interpretations can be proposed for this behavior: (i) exchange broadening due to O_2 exchange; (ii) broadening due to unresolved $^1J_{^{17}O-^{17}O}$ coupling constant; (iii) two possible conformational states of the $Fe-O_2$ moiety, as suggested originally by Spartalian et al.,[16] which are characterized by a small energy separation and very different electric field gradient tensors at the oxygen sites. Since, according to eq 1 the ^{17}O line widths are directly proportional to $\chi^2(1 + \eta^2/3)$, it can be anticipated that changes in this parameter can dominate the observed variations in line widths.

O_2 exchange can influence the effective relaxation of the ^{17}O nucleus. If the lifetime τ_e of the nucleus O(i) (i = 1 or 2) in the diamagnetic site is sufficiently large compared with the inverse of the difference in resonance frequencies between the bound state and free paramagnetic molecular oxygen, then the measured relaxation rate of O(i), $T_2^{*-1}[O(i)]$, is given by

$$T_2^{*-1}[O(i)] = T_2^{-1}[O(i)] + \tau_e^{-1} \qquad (2)$$

where $T_2^{-1}[O(i)]$ is the relaxation rate in the complex in the absence of exchange. Since $T_2^{-1}[O(i)] = T_1^{-1}[O(i)]$, one obtains

$$T_2^{*-1}[O(i)] = T_1^{-1}[O(i)] + \tau_e^{-1} \qquad (3)$$

We measured longitudinal $T_1[O(2)]$ relaxation times at 263 and 298 K. The resulting values (0.88 ms, estimated error ~5%, and 0.32 ms, estimated error ~10%, respectively) were found to be very similar to the corresponding $T_2^*[O(2)]$ values (0.84 and 0.29 ms, respectively), calculated from line-width measurements (Table I). Since no difference between $T_1[O(2)]$ and $T_2^*[O(2)]$ is observed, one can conclude that exchange processes play a negligible role in the ^{17}O line widths.

Let us consider now the effects of line broadening due to $^1J_{^{17}O-^{17}O}$ coupling constant when using gaseous oxygen containing 50% oxygen-17. The probability ϵ of a double labeling as that of direct coupling is therefore 25%. If the relaxation times of O(2) and O(1) are long compared to the $^1J_{^{17}O-^{17}O}$ coupling constant, the spectrum of both oxygen nuclei is a symmetric sextuplet, each component having the same intensity but unequal line widths,[38] superimposed on a much stronger central peak due to uncoupled nuclei. For very short relaxation times, the nuclei are fully decoupled. For intermediate $T_1[O(1)]$ values a more or less broadened component of the O(2) spectra should be observed. The intensity of the broadened component should also depend on the isotopic enrichment of the oxygen sites. Additional experiments with $^{17}O_2$ gas enriched at 70% (ϵ ~50%) and 33% (ϵ ~ 11%) showed no difference in the line shape. Therefore it can be concluded that the effect of scalar relaxation is negligible.

The above considerations leave interpretation iii as the only source of the temperature-dependent line-width anomalies. According to X-ray structural data of (O_2)FeTpivPP(1-MeIm),[9,24] the 1-MeIm is 2-fold disordered with respect to the orientation of the *N*-methyl group and the Fe–O–O plane is 4-fold statistically disordered, bisecting the N–Fe–N right angles of the equatorial porphyrin plane. Two of the four positions that the terminal oxygen atom can occupy are crystallographically independent. In one of these, orientation A, the Fe–O–O plane is approximately parallel to the trans axial base plane and in the other, orientation B, it is approximately normal to it. The dihedral angle between the alternative orientations of the Fe–O–O plane is 90°. Jameson and Drago[40] have recently performed CCF calculations based on the more accurate structural data of (O_2)FeTpivPP(2-MeIm)·EtOH.[41] In orientation B, the N(H)··O(1) distance is 3.88 Å and CH_3···O(1) contacts are 2.98 Å. In orientation A, the N(H)··O(1) distance is 4.19 Å and CH_3···O(1) contacts are 2.77 Å. Jameson and Drago[40] concluded that although N(H)···O(1) distances are ~1 Å longer than conventional hydrogen bonds and the N(H)···O(1) atoms adopt a not exactly linear arrangement, there is a substantial net attraction between the dioxygen moiety and the amide groups and smaller repulsive interactions between the oxygen molecule and the methyl groups which lower the net attraction. Only when the contacts between O_2 and the four surrounding NH CO moieties are considered is an energy of −9.5 kcal/mol calculated for both orientations of the disordered dioxygen ligand. For the complete pivalamido groups, the net attraction decreases to −7.9 kcal/mol (orientation A) and −5.2 kcal/mol (orientation B), due to repulsive terms between the dioxygen and methyl groups.

Our ^{17}O line-width data provide the first direct evidence that the $Fe-O_2$ moiety undergoes a conformational excitation accompanied by a dramatic change of the electric field gradient tensors around each oxygen nucleus, especially that of O(2). This conformation excitation involves purely diamagnetic states as can be shown by the absence of contact, temperature-dependent, paramagnetic chemical shifts and line widths. The existence therefore of a temperature-dependent $Fe(d^6)O_2 \leftrightarrow Fe(d^5)O_2^-$ equilibrium[18] or of a thermally populated triplet paramagnetic excited state[2,18] should be excluded. The energy separation of the two states is, according to Jameson and Drago,[40] 2.7 kcal/mol. This corresponds to population ratios, given by the Boltzmann factor $P_{II}/P_I = \exp(-E_0/kT)$, of 0.005 and 0.010 at 253 and 303 K, respectively (P_I corresponds to the low-energy conformer). The small difference in the population ratios between the two temperatures cannot account for the dramatic change observed in the oxygen-17 line widths. Spartalian et al.[16] calculated an energy difference of 0.29 kcal/mol, on the basis of zero-field Mössbauer spectra, which corresponds to population ratios of 0.56 and 0.62 at 253 and 303 K, respectively, the difference being rather small to account for the change in the ^{17}O line width. Accurate estimation of ΔE, which seems to be smaller than the latter value, requires temperature values at which the oxygen-17 line-widths ratios reached their low- and high-temperature constant values, which is not case as can be seen in Table I. Further studies are now in progress with a variety of low-viscosity and high-boiling-point solvents in an effort to extent the temperature range of the ^{17}O NMR measurements.

In compounds **5** and **6**, contrary to (O_2)FeTpivPP(1-MeIm) (**2**), the ratio of the line widths of both oxygen sites is practically temperature independent. This can be attributed to the existence of a major conformer significantly stabilized by hydrogen-bonding interactions with the distal NH of the handle (Lavalette et al.[14] suggested a gain in free energy of ~1.3 kcal/mol for the amide–BHP relative to the ether–BHP, while Jameson and Drago[40] calculated an energy gain of 14 kcal/mol for an N(H)···O(1)

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[36] The asymmetry parameter $\eta = (q_{xx} - q_{yy})/q_{zz}$, where q_{xx}, q_{yy}, and q_{zz} are the principal components of the electric field gradient tensor, has a value between 0 and 1.

[37] The slow-exchange condition is very probably fulfilled since the NMR signal of the paramagnetic free molecular oxygen, $^{17}O_2$, is expected to be considerably shifted by interaction of the magnetic moment of the nucleus and that of the unpaired electrons (hyperfine interactions). Detection of this signal should require special experimental techniques due to its extremely broad line width (Dundon, J. M. *J. Chem. Phys.* **1982**, *76*, 2171–2173).

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[39] Reference deleted in press.

[40] Jameson, G. B.; Drago, R. S. *J. Am. Chem. Soc.* **1985**, *107*, 3017–3020.

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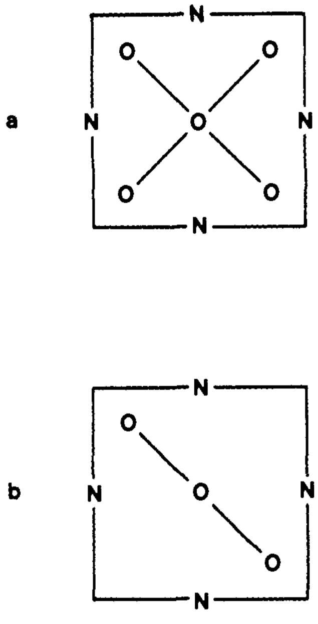

Figure 2. Schematic presentation of the Fe–O–O plane in (a) 1-MeIm picket-fence porphyrin and (b) amide–BHP.

separation of 3.0 Å). An interesting consequence of the above conclusions is that the bent oxygen ligand remains confined in these compounds within one single plane passing through the meso positions at which the distal handle is anchored in contrast with the 4-fold disorder observed for the picket-fence porphyrin (Figure 2). It should be emphasized that in the latter compound the interconversion rate is sufficiently rapid for the effective chemical shifts to be averaged since $T_1 = T_2$. This implies a low energy barrier for rotation around the Fe–O axis.

According to the ^{17}O NMR line-width data the two different types of FeO$_2$ conformation give rise to significantly different electric field gradient tensors at both oxygen sites, especially that of O(2). The magnitude of $\chi^2(1 + \eta^2/3)$ is dependent on the ground-state charge distribution around the oxygen nucleus. However, in contrast to ^{17}O chemical shifts, this parameter has found very limited applications as indicator of changes in the electronic environment for a given functional group. Detailed ^{17}O NQR[42,43] and NMR[44] studies showed that nuclear quadrupole coupling constants are dependent on the individual H-bond geometry and strength, the changes being usually between 7 and 13% even for molecules with intramolecular hydrogen bonds with very short O···O distances and strong deviations from linearity. It can therefore be concluded that the large changes of the electric field gradient tensors around each oxygen nucleus cannot be primarily due to long-range dipole–dipole interactions which should be expected to affect mainly the terminal oxygen O(1) rather than O(2), contrary to the experimental data. Such a dramatic change of the electric field gradient tensor at O(2) might result from a difference in the local symmetry due to differences in the M–O–O bond angle.[45] Jameson and Drago noted that in the maximization of the electrostatic attractions between the oxygen molecule and NH moieties, substantial distortions up to 25° of the M–O–O bond angles may occur. Interestingly, compound **3**, in which the superstructures are linked to the macrocycle by ether groups and thus no hydrogen bonding can occur, exhibits a ratio of the line widths of the two ^{17}O resonances that is similar to that of the amide compound **5**. Unfortunately, due to the low stability of the compound **3** it was not possible to perform variable-temperature experiments and relaxation time measurements to confirm the effect of exchange broadening on the line widths. Supposing that the effect of exchange broadening is minimal;[46] it can then be concluded from the ratio of the line widths (Table I) that the geometry of the Fe–O$_2$ moiety is substantially different from that of the conformers of the picket-fence porphyrin model.

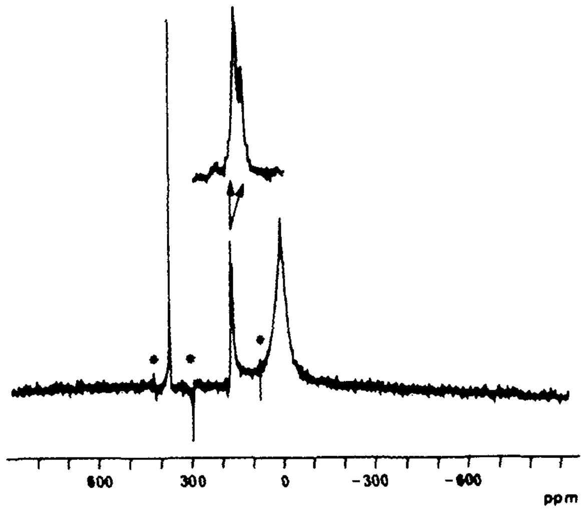

Figure 3. Low-frequency region of the ^{17}O NMR spectrum of compound **3** from use of the normal 90° pulse sequence. Concentration 1.8×10^{-2} M in CH$_2$Cl$_2$, $T = 273$-K, acquisition time $T_{acq} = 10$ ms, number of scans ~27 000, line-broadening filtering LB = 50 Hz, total experimental time ca. 45 min. Note the imperfect phasing due to the preacquisition delay used ($\Delta t = 50$ μs). The asterisk denotes spurious signals.

Further studies are currently in progress with a variety of bulky substituents on the handles in order to analyze the relative importance of the dioxygen/amide and dioxygen/methyl group interactions and the effect on the Fe–O–O bond angle. Nevertheless, three important conclusions can be drawn from the present data:

(i) The Fe–O$_2$ moiety of the picket-fence porphyrin model [(O$_2$)FeTpivPP(1-MeIm)] undergoes a conformational excitation accompanied by a significant modification of the electric field gradient tensor at both oxygen sites, especially that of O(2).

(ii) Change in the Fe–O–O bond angle is supposed to be the primary cause of this observed dramatic change.

(iii) The longstanding enigma of the electronic nature of the excited state is solved in favor of a purely diamagnetic state.

Oxidation Mechanism of Oxygen Adducts. It is known that the stability of the oxygenated species of iron porphyrin compounds is largely dependent on the concentration and the nature of additional ligands and on the concentration of protons in the solution. With the single-face hindered complexes, such as picket-fence porphyrin **2** and the so-called hybrid porphyrin **5**, the bulky substituents protect one side of the heme in such a way that no irreversible autoxidation takes place as long as the other side is occupied by an axial ligand that favors reversible oxygenation. But at low concentration of imidazole, the undesired μ-oxo dimer formation can still take place on the unprotected side of the heme. The steric encumbrance of both faces in basket-handle porphyrins inhibits this oxidation mechanism because the bimolecular reaction pathway via μ-peroxo dimer formation is prevented.[4]

The ultimate oxidation products in toluene or methylene chloride solutions of compounds **1**, **3**, and **4** always exhibit optical and ESR spectra characteristic of monomeric porphyrin Fe^{III}OH$^-$ derivatives.[47] The lifetime of these oxygenated derivatives is strongly dependent of the concentration of water in the solution. For example, the lifetime of compound **4** was approximately 0.25 and 1 h in water-saturated and anhydrous toluene, respectively,

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(42) Cheng, C. P.; Brown, T. L. *J. Am. Chem. Soc.* **1979**, *101*, 2327–2334.

(43) Butler, L. G.; Brown, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 6541–6549.

(44) Jaccard, G.; Lauterwein, J. *Helv. Chim. Acta* **1986**, *69*, 1469–1485.

(45) An alternative interpretation would be an elongation of the Fe–O bond due to anharmonic vibrations of the oxygen molecule in the iron binding potential well. Such an elongation would result in a low-frequency ^{17}O NMR chemical shift in agreement with the experimental results (Table I); however, it is not expected to affect drastically the symmetry and, therefore, the electric field gradient tensor at the oxygen bound to iron.

(46) Ether–BHP exhibits a higher dissociation rate relative to that of amide–BHP.[4] Therefore caution is necessary in interpreting ratios of line widths purely in terms of ratios of the parameter $\chi^2(1 + \eta^2/3)$.

(47) Lexa, D.; Momenteau, M.; Saveant, J. M.; Xu, F. *Inorg. Chem.* **1985**, *24*, 122–127.

at 25 °C.[14] In this work, we attempted to analyze the complicated oxidation mechanism of the oxygenated compound **3** in water-saturated methylene chloride. During the autoxidation process, several resonances of oxygen-17 enriched species were detected at low frequencies (Figure 3). The resonance absorption at $\delta = 11.1$ ppm ($\Delta\nu_{1/2} = 2080$ Hz) is characteristic of $H_2{}^{17}O$, and two sharp resonances at $\delta = 168.5$ and 174.3 ppm can be assigned to $H_2{}^{17}O_2$. The two latter peaks must correspond to hydrogen peroxide in two different environments. Only the resonance at 174.3 ppm is observed for $H_2{}^{17}O_2$ dissolved in dry toluene. The second peak at 168.5 ppm is then probably due to $H_2{}^{17}O_2$ in microscopic water droplets in suspension, which are always present in organic solutions when a two-phased preparative system is used.

From the presence of enriched water and hydrogen peroxide formed during the oxidation process, the following reaction scheme may be proposed:

$$\mathrm{PFe^{II}} + {}^{*}\mathrm{O_2} \rightleftharpoons \mathrm{PFe^{II}}\text{–}{}^{*}\mathrm{O_2}$$

$$\mathrm{PFe^{II}}\text{–}{}^{*}\mathrm{O_2} + \mathrm{H_2O} \rightarrow [\mathrm{PFe^{III}}\cdots{}^{*}\mathrm{O_2}^{\bullet-}\cdots\mathrm{HOH}]$$

$$[\mathrm{PFe^{III}}\cdots{}^{*}\mathrm{O_2}^{\bullet-}\cdots\mathrm{HOH}] \rightarrow \mathrm{PFe^{III}}\text{–}\mathrm{OH^-} + \mathrm{H}{}^{*}\mathrm{O_2}^{\bullet}$$

$$2\mathrm{H}{}^{*}\mathrm{O_2}^{\bullet} \rightarrow \mathrm{H_2}{}^{*}\mathrm{O_2} + {}^{*}\mathrm{O_2}$$

$$\mathrm{H_2}{}^{*}\mathrm{O_2} \rightarrow \mathrm{H_2}{}^{*}\mathrm{O} + \tfrac{1}{2}{}^{*}\mathrm{O_2}$$

This scheme seems to be in agreement with the autoxidation of hemoproteins.[48,49] Indeed, the replacement of histidine E7 in the α-chains of HbA by a potentially more strongly H-bonding residue, tyrosine (as in HbM Boston), leads to easier autoxidation probably due to H bonding to molecular oxygen strong enough to liberate a superoxide anion.[50] In the model compounds, it seems reasonable that water allows the proton-driven oxidation to form a labeled hydrosuperoxide radical that gives $H_2{}^*O_2$ and $H_2{}^*O$ in further reactions.

The NMR spectrum in methylene chloride also exhibits a sharp resonance at 372.9 ppm ($\Delta\nu_{1/2} = 108$ Hz) that has not yet been assigned confidently. It could be due to an oxidation reaction of the solvent molecules since no such resonance is observed when toluene is used as a solvent.

^{17}O NMR appears therefore of great potential value for the analysis of both the nature and the reactivity of the Fe–O_2 moiety in hemoprotein models.

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Consistent Porphyrin Force Field. 3. Out-of-Plane Modes in the Resonance Raman Spectra of Planar and Ruffled Nickel Octaethylporphyrin

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Abstract: Low-temperature (12 K) resonance Raman spectra are reported for tetragonal and triclinic crystallites of nickel octaethylporphyrin and its meso-d_4, $^{15}N_4$, and methylene-d_{16} isotopomers. Spectral contributions of the A and B forms in mixed triclinic crystals have been isolated by computer subtraction. The low-frequency (<900 cm^{-1}) spectra contain contributions from in-plane and out-of-plane porphyrin modes. With the aid of a normal-coordinate calculation, a majority of the out-of-plane modes have been assigned and described in terms of local-coordinate contributions. Modes are identified that involve cooperative motions of the pyrrole ring, i.e., tilting, folding, swiveling, and translation. These are implicated in static and dynamic distortions of porphyrin molecules. The ethyl bending coordinates have a major perturbing effect on the out-of-plane frequencies, which will have to be taken into account in extending the analysis to heme proteins. Tentative out-of-plane assignments are also given for nickel tetraphenylporphyrin.

In this paper we develop the first out-of-plane force field for the porphyrin ring based on experimental data. The out-of-plane potential surface is important with respect to the dynamical properties of heme proteins. The heme group is embedded in a protein pocket and is subject to a variety of forces that can distort it from planarity. Such distortions are readily seen in heme protein crystal structures. It is important to know what the restoring forces are in the porphyrin skeleton and how the out-of-plane motions affect the dynamics of, e.g., ligand binding or electron transfer.

The resonance Raman (RR) spectrum is potentially a source of information about these motions,[1] and indeed heme proteins generally display richly detailed RR spectra in the low-frequency region, which may contain bands due to out-of-plane (oop) modes. Marked variations in this region are seen among different proteins, which may well reflect different oop distortions. Thus it is important to have reliable oop assignments to analyze these differences.

The task of assignment is difficult because in the absence of symmetry-lowering effects these modes generally do not appear in the RR spectra. In the ideal D_{4h} symmetry of a metalloporphyrin only the E_g oop modes are Raman allowed (most are "u" modes), and their resonance enhancement requires vibronic

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