^{17}O Nuclear Magnetic Resonance Chemical Shift in Oxyhaemoglobin

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The ^{17}O chemical shift of oxygen in oxyhaemoglobin is calculated for two models, one corresponding to the Griffith structure and the other to the Pauling structure. In both cases the oxygen resonance is predicted to be several thousand ppm to low field of the oxygen resonance in water. The shift between the oxygen nuclei in the Pauling structure is predicted to be at least one thousand ppm. This large deshielding arises from the local environment of the oxygen molecule and depends critically on the splitting of the degenerate π orbitals on complexing.

INTRODUCTION

Precise details of the geometry and electronic structure of the oxygen-iron bond in oxyhaemoglobin are not yet known. A structure in which the axis of the oxygen molecule is parallel to the plane of the haem group has been proposed by Griffith,[1] and Pauling[2] has discussed a skew arrangement. In both these structures the complex is diamagnetic because the degeneracy of the oxygen π orbitals is lifted. Recently[3,4] it has been suggested that both iron and oxygen portions may be in triplet states and the overall diamagnetism arises from spin pairing of these. This would allow an axially symmetric structure but there seems no reason why such a structure should have a singlet ground state rather that the quintet ground state predicted by Hund's rules. In order to obtain more experimental information about the structure of the oxygen in oxyhaemoglobin Maričić, Leigh and Sunko[5] have attempted to measure the ^{17}O chemical shift of isotopically enriched oxyhaemoglobin. Unfortunately it has not yet proved possible to detect the signal from the bound oxygen[6], but when successful this experiment will give useful information. In particular if two signals are observed the Griffith model would be ruled out.

In this paper we present the results of some calculations of the ^{17}O chemical shift in the molecule. Maričić et al.[5] used the methods of Karplus and Das[7] to estimate the local paramagnetic contribution to the shielding. However in another paper[8] we show that it is probable that the use of an average energy approximation (as in the method of Karplus and Das) is particularly bad for ^{17}O chemical shifts, and that reasonable estimates can be obtained from an extended Hückel method. This is particularly true for oxyhaemoglobin as the local paramagnetic term is dominated by transitions

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between the two antibonding π orbitals which are degenerate in the free molecule.

1. *Method of Calculation*

In these calculations we used the method and parameters we have proposed[8] for oxygen chemical shift calculations. Essentially this method is an extended Hückel calculation of the type proposed by Pople and Santry[9] in which molecular orbitals are calculated as linear combinations of the valence shell atomic orbitals ($2s$ and $2p$ oxygen) and the paramagnetic shielding evaluated for all possible electronic excitations within this restricted set of orbitals.

It is impracticable to treat the complete oxyhaemoglobin molecule in this way. However, as it seems probable that the modifications to the molecular wave functions of O_2 on complexing are comparatively small and that significant electron transfer between oxygen and iron does not occur, we can consider the O_2 ligand as an independent unit. The effect of complexing on the electronic structure of O_2 is described by means of a complexing field (the converse of a ligand field) which lowers the energies of some p atomic orbitals relative to others. We considered two models for this interaction corresponding to the Griffith and Pauling structures and calculated the local contribution to the chemical shift. In addition we estimated the long range contributions of the porphyrin ring and the iron atom to the screening of the oxygen. These are almost negligible; the values are given in section 3.

In model A, which corresponds to the Griffith structure (Fig. 1a) both oxygen atoms remain equivalent but the energies of the p_x atomic orbitals are lowered relative to the p_y orbitals (the z direction is defined along the molecular axis).
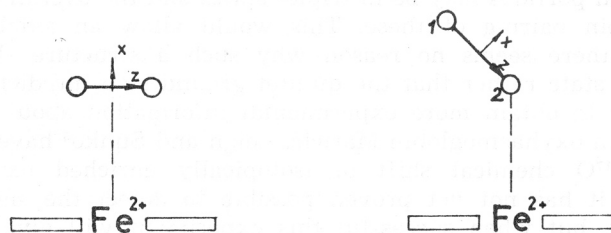

Fig. 1. Models for the structure of oxyhaemoglobin
a) Model A: Griffith structure
b) Model B: Pauling structure

It is more difficult to produce a simple model corresponding to the Pauling structure, as the atoms are inequivalent, and both p_z and p_x orbitals of the atom nearest the iron are affected (Fig. 1b). Model B is a single parameter description of this situation in which all p orbitals have the same energy except p_z and p_x of the near oxygen atom, which are assumed to be equally stabilised by the complexing field. In this model the degeneracy of the π orbitals is lifted as in model A, and, in addition, electron drift occurs within the ligand. This model obviously predicts two chemical shift values, one for each oxygen atom.

Molecular orbital functions were constructed from $2s$ and $2p$ oxygen atomic orbitals. All overlap terms were neglected and the Mulliken approximation

$$\beta_{ij} = \tfrac{1}{2}(\alpha_i + \alpha_j)S_{ij} \qquad (1)$$

was used for the resonance integrals. The overlap integrals, S_{ij}, in this expression were calculated for Slater orbitals with exponents $\varrho(2s) = 7.7$, $\varrho(2p) = 2.275$ and an internuclear distance of 1.207Å. The following values of the coulomb integrals α_i were used (Δ is the effect of the complexing field).

Model A	Model B
$\alpha_s = -35.3\,\mathrm{eV}$	$O_1 : \alpha_s = -35.3\,\mathrm{eV} \; \alpha_p = -16.12\,\mathrm{eV}$
$\alpha_{p_z} = -16.12\,\mathrm{eV}$	$O_2 : \alpha_s = -35.3\,\mathrm{eV} \; \alpha_{p_x} = -16.12\,\mathrm{eV}$
$\alpha_{p_x} = -16.12 - \Delta/2\,\mathrm{eV}$	$\alpha_{p_y} = \alpha_{p_z} = -16.12 - \Delta\,\mathrm{eV}$
$\alpha_{p_y} = -16.12 + \Delta/2\,\mathrm{eV}$	

For each model eight molecular orbitals are obtained from this restricted basis set; in the ground state the six lowest are filled and two highest empty. The chemical shifts of both oxygen atoms were calculated by the method described elsewhere[8] in which the infinite sum in the Ramsey formula (2) for the shielding

$$\sigma_N = \sigma_0 \left\{ \langle o \,|\, \frac{1}{r_N} \,|\, o \rangle + \frac{1}{2} \sum_n (E_0 - E_n)^{-1} \langle o \,|\, \Sigma_k l_{kn} r_{kn}^{-3} \,|\, n \rangle \cdot \langle n \,|\, \Sigma_k l_{kn} \,|\, o \rangle + \text{c. c.} \right\} \quad (2)$$

is approximated by including only the twelve excited states obtained by single electron excitations to the two empty molecular orbitals constructed from the valence shell.

2—1 Results for Model A

Table I shows the results of the calculations for model A. The energies of the molecular orbitals for different values of Δ are shown, followed by the splitting Δ' between the x and y antibonding π orbitals and the calculated shielding constant σ. This includes both local diamagnetic and local paramagnetic parts, but not the contribution of about -100 ppm due to the long range terms (see section 3). The value in the table is relative to an oxygen atom stripped of its valence electrons. Experimental measurements are usually referred to $H_2{}^{17}O$ which is calculated[8] to have a shift of $+$ 120 ppm on this scale.

In Fig. 2, chemical shift values are plotted against Δ', the separation of the π_{gx} and π_{gy} molecular orbitals. As long as Δ, the splitting of the atomic orbitals, is less than about 5eV this is just the difference in energy between the lowest empty and highest filled molecular orbitals. In order for the ligand to be diamagnetic Δ' must be at least as large as the exchange energy of the triplet ground state of O_2, which is about 1eV.[10] At this value the shift is about $-3,000$ ppm and even if Δ' were as big as 4eV the shift is calculated to be $-1,000$ ppm. This represents a reasonable range of values of the complexing field.

TABLE I

Energies of Molecular Orbitals (E) and Chemical Shifts (σ) Calculated for Model A

Δ ev	E(σ_g) ev	E(σ_u) ev	E(π_u)_x ev	E(σ_g*) ev	E(π_u)_y ev	E(π_g*)_x ev	E(π_g*)_y ev	E(σ_u*) ev	Δ' (E_{πgy}* − E_{πgx}*) ev	σ ppm
0.50	−47.54	−29.09	−18.77	−18.66	−18.20	−13.97	−13.55	−7.55	0.42	−6133
1.00	−47.54	−29.09	−19.06	−18.66	−17.91	−14.19	−13.33	−7.55	0.86	−3262
1.50	−47.54	−29.09	−19.34	−18.66	−17.62	−14.40	−13.12	−7.55	1.28	−2292
2.00	−47.54	−29.09	−19.63	−18.66	−17.34	−14.61	−12.90	−7.55	1.71	−1798
2.50	−47.54	−29.09	−19.92	−18.66	−17.05	−14.83	−12.69	−7.55	2.14	−1496
3.00	−47.54	−29.09	−20.20	−18.66	−16.76	−15.04	−12.48	−7.55	2.56	−1290
3.50	−47.54	−29.09	−20.49	−18.66	−16.48	−15.25	−12.26	−7.55	2.99	−1140
4.00	−47.54	−29.09	−20.78	−18.66	−16.19	−15.47	−12.05	−7.55	3.42	−1025
5.50	−47.54	−29.09	−21.64	−18.66	−15.33	−16.11	−11.41	−7.55	4.70	−801

TABLE II

Energies of Molecular Orbitals (E), Charge Distribution (q) and Chemical Shifts (σ) Calculated for Model B

Δ ev	E(σ_s) ev	E(σ_s*) ev	E(σ_p) ev	E(π_x) ev	E(π_y) ev	E(π_x*) ev	E(π_y*) ev	E(σ_p*) ev	Δ' (E_{πy}* − E_{πx}*) ev	q e	σ_1 ppm	σ_2 ppm
0.50	−47.59	−29.15	−18.94	−18.78	−18.48	−13.96	−13.76	−7.66	0.20	0.029	−13216	−11844
1.00	−47.64	−29.21	−19.22	−19.11	−18.48	−14.13	−13.76	−7.76	0.37	0.056	−7513	−6108
1.50	−47.70	−29.28	−19.50	−19.45	−18.48	−14.29	−13.76	−7.86	0.53	0.082	−5607	−4171
2.00	−47.75	−29.35	−19.82	−19.78	−18.48	−14.42	−13.76	−7.95	0.66	0.107	−4653	−3186
3.00	−47.87	−29.51	−20.61	−20.35	−18.48	−14.63	−13.76	−8.11	0.87	0.153	−3699	−2175
4.00	−48.00	−29.69	−21.44	−20.90	−18.48	−14.80	−13.76	−8.25	1.04	0.193	−3224	−1647
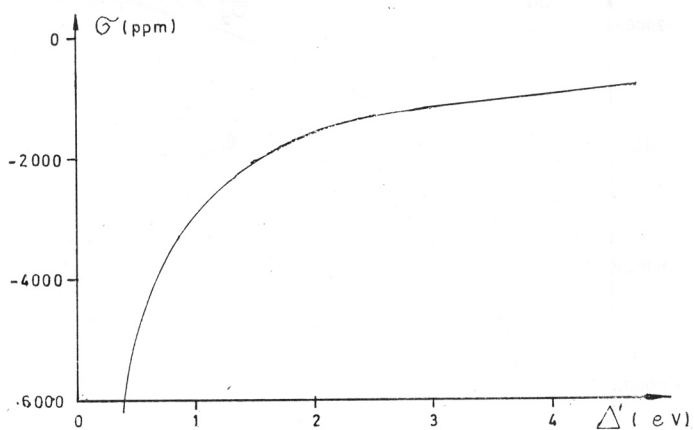
Fig. 2. Calculated ^{17}O shift σ for model A as a function of the energy difference of the π_g orbitals, Δ'. The shielding in water is $+$ 120 ppm on this scale.

If σ is plotted against $(\Delta')^{-1}$ a good straight line is obtained showing that the calculated shielding for this model is dominated by the π_{gx} to π_{gy} excitation. In free O_2 these orbitals are degenerate. The large deshielding of the oxygen relative to a bare nucleus can readily be understood. The state of free O_2 which corresponds to the configuration of the ligand O_2 in this model is a singlet with both antibonding electrons in the same π_g orbital. As such a state is orbitally degenerate and consequently paramagnetic, the field at the nucleus would be enhanced by the electronic fields. If this degeneracy is lifted by a small amount the molecule remains paramagnetic, but the paramagnetism is now temperature independent and is due to mixing of the orbitals which were previously degenerate. The nucleus is still deshielded.

2—2 Results for Model B

The molecular orbitals energies, shielding constants and atomic charges calculated for model B for various values of Δ, are shown in Table II. In this model the separation Δ' of the lowest empty (π_y) and highest occupied (π_x) orbitals is much less than in model A. The calculated shifts of the two atoms are shown plotted against Δ in Fig. 3. Again both nuclei show a large low field shift, but there is also a considerable splitting between the two. Nucleus 2, near the iron ion, which bears the negative charge q shown in Table II, is always less shielded than the other nucleus by about 1400 to 1500 ppm. This shift between the two nuclei is remarkably constant and independent of the value of the charge q. In this calculation the dependence of $\langle r^{-3} \rangle$ on charge was taken into account.

3. Long Range Contributions

The total shielding of the oxygen atoms includes contributions from the protein, the porphyrin ring and the iron atom in addition to the effects of the local electronic environment calculated in the previous sections. Of these effects the only two parts which are likely to make significant contributions are the effects of ring currents in the porphyrin and the effects of the d electrons on the iron.
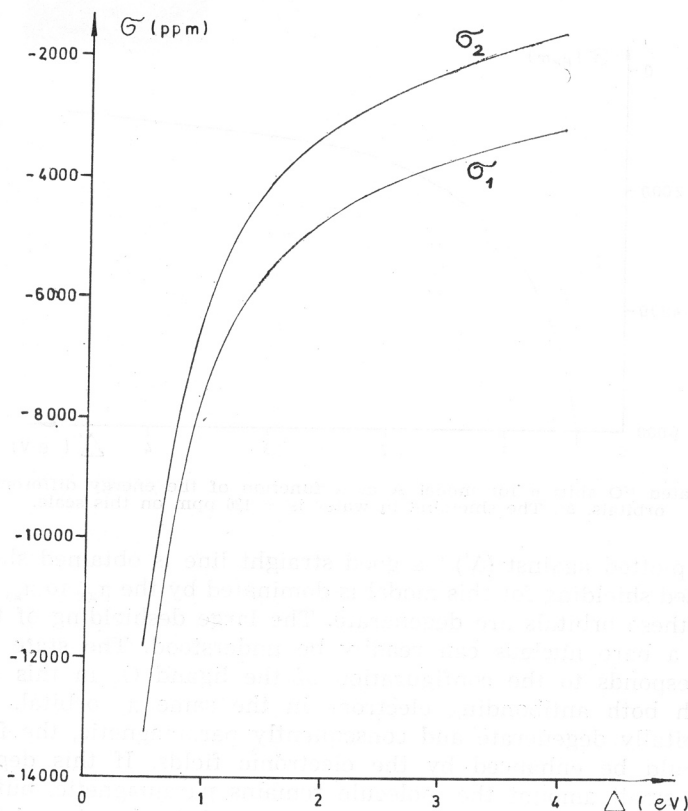
Fig. 3. Calculated ¹⁷O shifts for nuclei 1 and 2 in model B as a function of the complexing field Δ.

The contribution from the porphyrin ring has been estimated by Maričić et al.[5] to be about + 10 ppm. We have confirmed this estimate for an oxygen atom at about 2Å from the iron atom. This is only the same order of magnitude as the present experimental error in measuring oxygen chemical shifts and is considerably less than the uncertainties in our calculation.

There is a slightly larger contribution from induced currents in the d orbitals of the iron atom. These contribute an amount to σ: —

$$\sigma^N(Fe) = -\sigma_o \frac{1}{2} \sum_{i,j} \left\{ (\epsilon_j - \epsilon_i)^{-1} \langle i | l_N | j \rangle \cdot \langle j | l_N r_N^{-3} | i \rangle + c.\ c. \right\} \quad (3)$$

In this expression the summation is over occupied (i) and empty (j) d orbitals of iron, but the angular momentum hl_N and distance operator r_N are relative to the oxygen nucleus. In order to evaluate this we need the energies of the d orbitals in iron. These are not known for oxyhaemoglobin with any certainty. We used the values given by Zerner et al.,[11] and assumed that the electrons were localised in the d orbitals. Any delocalisation into the porphyrin ring will reduce the effect. The values of r_N^{-1} and r_N^{-3} (needed to estimate the diamagnetic contribution) were calculated for an oxygen nucleus

2.06 Å above the iron atom using best two exponent Slater d functions.[12] The contribution to the screening of this nucleus (1) in model B is

$$\sigma(Fe) = -100 \text{ ppm}$$

As this term falls off rapidly with distance, the further nucleus (2) is not affected by it.

The oxygen nuclei in model A are slightly further from the iron (2.15 Å) and the contribution to the screening is

$$\sigma(Fe) = -83.5 \text{ ppm}.$$

Both these figures are small compared to the large low field shifts due to the local electronic environment of oxygen and are similar to the uncertainties of the calculation.

DISCUSSION

It is often difficult for an experimentalist to know how much faith to put in a theoretical calculation; he may either expect agreement to the fourth significant figure or disbelieve the result altogether. This calculation is crude. Many approximations have been made and the reliability of the method can best be judged by other results to be published elsewhere.[8] We are confident that in either model the resonance of the oxygen nuclei will be at least 1,000 ppm to 4,000 ppm to low field of water. If the Griffith structure is correct then figure 1 shows the form of the relationship of σ and Δ' to an accuracy of a few hundred ppm. If however, the Pauling model is correct model B is rather crude as it contains only a single parameter to describe an asymmetric situation. The predicted shift difference (1500 ppm) of the two nuclei is likely to be correct to $\pm$ five hundred ppm, and would probably be enhanced by electron delocalisation.

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IZVOD

Kemijski pomak nuklearne magnetske rezonancije ^{17}O oksihemoglobina

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Kemijski pomak maksimuma za nuklearnu magnetsku rezonanciju oksigena (^{17}O) u oksihemoglobinu izračunat je za model u kojem je molekula oksigena paralelna porfirinskom prstenu hema, kao i za model u kojem je os oksigenove molekule pod kutem od 30⁰ na tu plohu. U oba slučaja izračunato je da bi rezonancija ^{17}O imala biti za nekoliko tisuća ppm na nižem polju od one u molekuli vode. Razlike u kemijskom pomaku između dviju rezonancija od asimetričnog modela iznose oko 1500 ppm.

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