SIGNIFICANCE OF THE ORIENTATION OF COORDINATED IMIDAZOLE AND BENZIMIDAZOLE GROUPS IN HAEMOPROTEIN AND VITAMIN B\textsubscript{12} STRUCTURES*

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Details of the structure of the dioxygen complex, Co(bzacen)(py)O\textsubscript{2} [py = pyridine; bzacen = N,N'-ethylenbis(benzoylacetonimide), PhC(O–)=CHC-(Me)=NCH\textsubscript{2}-]\textsubscript{2}], a possible model compound for oxygenated haemoproteins (Rodley and Robinson 1972), have prompted a study of the metal–ligand bonding in this compound and related biological systems. It has already been noted (Rodley and Robinson 1972) that the orientation of the benzimidazole group in the vitamin B\textsubscript{12} structures resembles closely that found for the pyridine plane in Co(bzacen)(py)O\textsubscript{2} (Fig. 1), and the recently determined structure of ferricytochrome c (Dickerson et al. 1971) also has this specific arrangement where the base (imidazole) plane bisects the haem metal–nitrogen bonds. In addition, the vitamin B\textsubscript{12} model compound (Lenhert 1967), Co(dmg)\textsubscript{2}(py)(o-methylcarboxymethyl), (dmg = dimethylglyoxime) and a five-coordinate compound, Co(salen)(py) (Calligaris et al. 1970), which is related to the Co(salen) oxygen-carrying system, have base planes in the same bisecting position. While it is difficult to assess the orientation of the coordinated imidazole group in

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oxyhaemoglobin, calculations using the published coordinates for metmyoglobin (Watson 1969) show the imidazole plane to be displaced about $15^\circ$ from the bisecting position. However, the structure of this "inactive" form may not be an accurate indication of the situation in the deoxy and oxy Fe$^{II}$ forms. Although the general orientation of the single base plane in a closely bisecting position in these model and biological structures may in part or even to a large extent result from a minimization of steric interactions, we consider that the geometry of the metal–base linkage could nevertheless be an important factor in the unique bonding and electronic properties of these systems. For comparison it might be noted that the base planes in three (electronically different) bis-imidazole iron compounds (Prout and Wiseman 1964; Bowman, Gaughan, and Dori 1972; Collins, Countryman and Hoard 1972) are generally close to the bisecting position but in each case displaced to a certain extent.

Many studies indicate that $\pi$ bonding plays a key role in the biological activity of haemoprotein and B$_{12}$ systems and we originally considered (Rodley and Robinson 1972) that the orientation of the pyridine ring in Co(bzacen)(py)O$_{2}$ might be important for cobalt–pyridine $\pi$ bonding. However, a closer study of the bonding in this complex indicates that the pyridine ring is oriented in such a manner as to specifically eliminate the possibility of M–py $\pi$ bonding. Further it can be shown that metal $\rightarrow$ base $\pi$ bonding would have an adverse effect on such features of these systems as (1) $\pi$ bonding within the planar haem, corrin, or metal–Schiff base grouping (hereafter referred to as the "square planar grouping"), (2) the stabilizing effect of metal (d$_{\pi}$)$\rightarrow$O$_{2}(\pi^*)$ bonding in dioxygen compounds, and (3) the nucleophilic character of vitamin B$_{12}$ systems. All would be reduced by M $\rightarrow$ base $\pi$ bonding. The orientation of the base in the bisecting position enables it to act as a pure $\sigma$ donor by eliminating the possibility of metal–base $\pi$ bonding.

In attempting to establish these points we will consider the bonding in the Co(bzacen)(py)O$_{2}$ complex under the following headings:

1. the orientation of pyridine molecular orbitals with respect to metal d$_{\pi}$ orbitals;
2. $\pi$ bonding in the square planar grouping;
3. cobalt–dioxygen bonding.

1. Metal–Pyridine Bonding

Apart from the formation of a nitrogen $\rightarrow$ metal $\sigma$ bond from the overlap of nitrogen sp$^{2}$ and metal d$_{\pi}$ orbitals there is the possibility of overlap of pyridine $\pi$ and metal d$_{\pi}$ orbitals. For the pyridine group in the bisecting position there are two alternative arrangements of pyridine $\pi$ and metal d$_{\pi}$ orbitals [Figs. 2(b) and 2(c)], depending on the choice of axes. The most likely arrangement is (b) as it is this orientation of d orbitals (Fig. 1) that is necessary for Co $\rightarrow$ CO$_{2}$ $\pi$ bonding, considered important for the stabilization of the Co–O$_{2}$ linkage [cf. Section (3) below]. However, it can be seen that for Figure 2(b) [in contrast to 2(c)] none of the pyridine orbitals match d$_{\pi}$ orbitals [this can best be seen by superimposing the views given in Figures 2(a) and 2(b)]. Metal–pyridine $\pi$ bonding is therefore excluded in the most probable arrangement [Fig. 2(b)]. For Figure 2(c) $\psi_{1}, \psi_{2}, \psi_{4}$, and $\psi_{6}$ could combine with metal d$_{\pi}$ orbitals and any displacement of the pyridine plane from the bisecting position
of Figure 2(b) towards an eclipsed position (xz or yz plane) would also enable metal-pyridine π bonding to occur.

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\begin{array}{cccccc}
+ & + & + & + & + & + \\
C-N-C & C-N-C & C-N-C & C-N-C & C-N-C & C-N-C \\
+ & - & - & - & - & - \\
\psi_1 & \psi_2 & \psi_3 & \psi_4 & \psi_5 & \psi_6 \\
\end{array}
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(a)

Fig. 2.—(a)–(c) Cobalt and pyridine π orbitals: (a) end-on view of pyridine π orbitals showing signs associated with the nitrogen atom and its adjacent carbon atoms; (b) view down z of the cobalt d_{xz}, d_{yz} orbitals that give zero overlap with pyridine π orbitals; (c) view down z of d_{xz}, d_{yz} orbitals oriented to overlap with \psi_1, \psi_2, \psi_4, and \psi_6 of (a). (d) and (e) Combinations of bzacen \( p_z \) and cobalt d_{p} (d_{xz}, d_{yz}) orbitals corresponding to (b) and (c) (view down z). (f) and (g) Orientation of pyridine plane in the bzacen and dmg structures. (h) and (i) Combinations of dmg \( p_z \) and cobalt d_{p} (d_{xz}, d_{yz}) orbitals (view down z).
(2) Bonding in the Square Planar Grouping

Some indication of the effect that the orientation of $d_\pi$ orbital lobes has on $\pi$ bonding in the square planar grouping may be obtained from Figures 2(d) and 2(e). Simple overlap schemes, corresponding to the alternative orientations of the $x, y$ axes [Figs. 2(b) and 2(c)], are presented. As the nodal plane bisects the chelate rings in 2(e) this would probably give less satisfactory $\pi$ delocalization than 2(d) where the nodal plane bisects the C–C bond of the ethylene bridge and complete delocalization is maintained in each chelate ring. Arrangement 2(d) would be advantageous for $\pi$ electron transfer in six-coordinate systems like Co(bzacen)(py)O$_2$ [cf. Section 3] and this further suggests that 2(b) is probably the preferred orientation of $d_\pi$ orbitals. It is also of interest that the orientation of the pyridine ring in five-coordinate Co(salen)(py) (Calligaris et al. 1970) is identical, with respect to the planar grouping, to that in Co(bzacen)(py)O$_2$. Details of the square-pyramidal Co(salen)(py) structure (Calligaris et al. 1970) indicate the retention of $\pi$ bonding within the square planar grouping and this may be facilitated by the orientation of the pyridine group in such a way [Figs. 2(b) and 2(d)] that it does not compete with the planar group for $d_\pi$ electrons. Thus the orientation that minimizes metal–pyridine $\pi$ bonding [Fig. 2(b)] also appears to give the best match [Fig. 2(d)] of orbitals for $\pi$ delocalization in the square planar groupings of both the six- and five-coordinate systems discussed here.

![Molecular orbitals](image)

Fig. 3.—Molecular orbitals for linear representation of the bzacen chelate grouping

(N–C–C–C–O$^-$) (view perpendicular to z).

An alternative approach to the above analysis is to consider the overlap of $d$ orbitals with $\pi$ molecular orbitals of the ligand framework, (N–C–C–C–O$^-$), shown schematically in Figure 3. Arrangement (b), Figure 2, would allow overlap with three of the orbitals ($\psi_1$, $\psi_3$, and $\psi_5$) while the alternative arrangement [Fig. 2(c)] allows overlap with $\psi_2$ and $\psi_4$ only. The overlap of $\psi_1$, $\psi_3$, and $\psi_5$ from each five-membered chelate group would permit both Co($\pi$)–L and Co($\pi$)→L bonding depending on the availability of $d_\pi$ electrons. In the five-coordinate complex, Co(salen)(py), this is probably Co($\pi$)→L but in the six-coordinate O$_2$ compound Co($\pi$)–L is probably more important as the result of Co($\pi$)→O$_2$ bonding.

(3) Metal–Dioxygen Bonding

E.p.r. studies of 1 : 1 dioxygen complexes indicate that the bonding is effectively Co$^{III}$–O$_2^-$ (Hoffman, Diemente, and Basolo 1970). While the 126° Co–O–O bond
angle (Rodley and Robinson 1972) indicates that cobalt–oxygen σ bonding results from the overlap of cobalt dπ and oxygen sp2 orbitals, electron transfer from cobalt to oxygen is probably achieved by delocalization of electrons in cobalt dπ orbitals into the empty π* orbital of the dioxygen molecule. The particular orientation found in the complex produces good overlap of lobes of the oxygen antibonding orbital with metal dπ orbitals (Fig. 1). As mentioned above the matching of orbital signs requires the dπ orbitals to be oriented in the manner shown in Figure 2(b). With the pyridine group oriented the way it is there is no possibility of competing M-(π)-→N bonding. On the other hand it is possible for metal→dioxygen π transfer to be compensated for, at least in part, by M←(π)-L square planar group π bonding as dπ can overlap with both empty O2, π*, and filled Schiff base π orbitals. In this way electron charge transfer from the Schiff base to the dioxygen molecule can be achieved. Also the pyridine group is able to independently assist in the compensation of M→O2 charge transfer by donating electrons as a pure σ donor, in the orientation observed. This is probably an important additional factor in stabilizing the metal–dioxygen linkage. The decrease in the M–N(py) distance of 210 pm in Co(salen)py to 202 pm in Co(bzacen)pyO2 indicates an enhancement of σ bonding on oxygenation (Calligaris et al. 1970; Rodley and Robinson 1972).

Before discussing the broader implications of the bonding scheme proposed for Co(bzacen)(py)O2 it is necessary to consider the dimethylglyoxime model compound for B12 coenzyme structures, Co(dmg)(py)(o-methylcarboxymethyl) because the orientation of the pyridine ring appears to be different from that observed for Co(bzacen)(py)O2 [Figs. 2(f) and 2(g)]. Two alternative combinations of dπ and pπ orbitals [Figs. 2(h) and 2(i)] may be considered. Unlike the Co(bzacen) case the choice that has a nodal plane bisecting the chelate ring [Fig. 2(i)] would probably be preferred as it is more consistent with the strong hydrogen bonding between the two dmg groups observed (the O–O distance is 250 pm) than the arrangement shown in Figure 2(h). Thus the orientation of the pyridine ring observed is not necessarily inconsistent with the arguments presented for Co(bzacen)(py)O2. In both cases the pyridine ring appears to orient at right angles to the square planar group's π nodal plane.

Knowledge of the relative energies of pyridine, oxygen, Schiff base, and metal orbitals would enable a more rigorous analysis than that presented here. It is possible that the energies of the pyridine π and metal dπ orbitals are far enough apart to make metal–pyridine π bonding unlikely no matter how the pyridine is oriented and that steric interactions primarily determine the orientation of the base planes. However, the success of the qualitative bonding analysis presented here in rationalizing specific base orientations for a wide range of compounds indicates that the mode of coordination observed may nevertheless be significant in relation to electronic factors [such as those involved in the stabilization of the Co(bzacen)(py)O2 complex]. If this is the case this analysis may provide insight into the role of the bases in related biological systems. One may envisage the square planar grouping and one of the axial groups producing an extended π system that acts as a buffer for electronic variations in the rest of the molecule. The axial base group bonds so as not to interfere with this π electron mobility while providing further flexibility in lieu of its σ donor properties. This may be related, for example, to studies (Schrauzer and Deutsch 1969) of the
reactions of vitamin B$_{12}$s, cobaloximes, and related compounds which show that these systems are very highly nucleophilic and that axial bases that can accept charge via $\pi$-electron back-donation from cobalt reduce this nucleophilic property. For the oxygen-carrying proteins the significant electronic changes that occur on oxygenation [which may trigger the extensive conformational changes that occur in haemoglobin on oxygenation (Perutz 1970)] are possible because of the cooperative effects of the $\pi$ and $\sigma$ bonding properties of the square planar and base groups. Interechain interactions in the myoglobin (Watson 1969) and ferricytochrome $c$ structures (proximal imidazole–proline hydrogen bonding) that appear to hold the imidazole groups in a fixed position (Dickerson et al. 1971) further indicate the importance of base plane orientation. For the cytochromes the electron-carrying function may be related to the same type of electronic situation suggested for the B$_{12}$ and oxygen-carrying systems. The square planar haem group has coordinated to it a base (imidazole), specifically oriented for $\sigma$ bonding and an atom with $\pi$-bonding properties (the sulphur of the methionine group) that can form an extended $\pi$-bonded system with it, giving as before a flexible and versatile electronic system.

The question of the orientation of base planes in vitamin B$_{12}$ and haemoglobin systems was discussed some time ago by Williams (1961). However, metmyoglobin was stated to have the imidazole group oriented in an approximately eclipsed position with respect to the iron–nitrogen porphyrin bonds. Our calculations show this is not the case. Also, the only specific bonding effect considered was the effect of repulsion between filled metal $d_{\pi}$ orbitals and imidazole electrons. However, the suggestion by Williams (1961) that the cooperative effects of oxygen binding in haemoglobin may arise (in part) from changes in the steric arrangement of the imidazole group relative to the porphyrin plane merits further consideration. Our analysis of the bonding in these systems would suggest that it may be more important for the imidazole group to be oriented in a bisecting position in the oxy form than in the deoxy form. Thus a decrease in the iron–imidazole distance (which would increase the importance of minimizing metal–base $\pi$ interactions) and a change in the orientation of the imidazole group may be concomitant effects in the oxygenation process as suggested by Williams (1961).

Single crystal e.p.r. studies of myoglobin derivatives (Helcké, Ingram, and Slade 1968; Peisach, Blumberg, and Wyluda 1971) provide evidence for the influence of base plane orientation on the bonding in haem systems.

References
