Role of Weak Hydrogen Bonding in the Coordination of Dioxygen to Hemoproteins and Their Models

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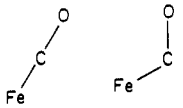
Abstract: The X-ray structural data on iron(II) "picket-fence" porphyrins have been reexamined in order to understand more clearly the interactions between coordinated dioxygen and carbon monoxide and the amide. The distances are long for normal hydrogen bonds but are consistent with calculations, indicating weak hydrogen bonding. When approximate CFF methods are used, substantial electrostatic interactions with the amide groups (O···(H)N ~ 4· Å) are observed; a −0.50-e⁻ net transfer of charge onto the O_2 ligand for Co relative to a −0.25-e⁻ transfer for Fe leads to a −9 kcal/mol greater attraction. These attractions are partially counterbalanced by repulsive interactions with the methyl groups of the pivalamide pickets (O···CH_3 ~ 2.8 Å). For the FeCO derivative, only weak interactions of the CO with both the amide and methyl moieties are calculated. Hydrogen bonding and the minimization of repulsive contacts with distal groups and the heme itself are probably important factors by which hemoglobins such as hemoglobin ascaris and leghemoglobin achieve dioxygen affinities several orders of magnitude greater than those for vertebrate hemoglobins such as myoglobin.

The substantial difference in the dioxygen affinity of planar unprotected cobalt(II) and iron(II) porphyrinato systems compared to vertebrate hemoglobins or "picket-fence" porphyrins[1] has puzzled chemists for some time.[1-3] This difference has been attributed in part to a differential solvation of the deoxy and oxy forms that is more pronounced and leads to lower dioxygen affinities for the former (unshielded) than for the latter (shielded) porphyrins[2] and in part to various polarity effects[4-7] for which evidence at room temperature has been equivocal.[3] The addition of a polar protic solute, 0.005 M CF_3CH_2OH, to a CH_2Cl_2 solution of a cobalt(II) pentadentate Schiff base complex Co(SMDPT) induced a dramatic 400-fold increase in dioxygen affinity at −26 °C.[6] More significantly, the enthalpy of dioxygen binding changed from −9.8 to −11.6 kcal/mol in the presence of trifluoroethanol. Since infrared studies clearly showed a hydrogen-bonding interaction between the alcohol and the bound O_2, the pronounced influence of hydrogen bonding on dioxygen affinity was demonstrated. The magnitude of the interaction, 6.6 kcal/mol, indicates a basicity for the coordinated dioxygen comparable to the oxygen atom of *N,N'*-dimethylacetamide and considerably less than that for a superoxide ion. Hydrogen bonding of O_2 to the distal imidazole in the proteins and to the N−H groups of the pickets was proposed to account for the increased values of the equilibrium constant for dioxygen binding in these systems.[6] Recently, FeO_2 and FeCO complexes of a tetrakis(orthoamidophenyl)porphyrinato derivative were prepared and characterized by NMR methods.[7] For the dioxygen complex, a 1 ppm shift in the amide proton resonances (relative to the CO and Zn complexes) was interpreted in terms of hydrogen bonding.

In the neutron diffraction study of MbO_2, the imidazole proton involved in the long proposed hydrogen bond to O_2[8] was located.[9] By way of contrast, in a parallel study of MbCO, the alternative imidazole tautomer is observed. No hydrogen bond exists, although contacts between the CO ligand and the imidazole group remain very close (2.7 Å).[10] Substantial tilting or displacement of the CO moiety from coincidence with the normal to the heme plane passing through the iron center is observed. Hydrogen



bonding to coordinated O_2 by either water or distal imidazole is further supported by X-ray diffraction studies of oxymyoglobin (MbO_2),[11] oxycobaltomyoglobin ($CoMbO_2$),[12] oxyerythrocruorin (ErO_2),[13,14] and oxyhemoglobin (HbO_2).[15] These observations are in accord with most notions that the FeO_2 group is substantially polarized, bestowing a partial negative charge on O_2, whereas the FeCO group is not extensively polarized.

In a recent study the dioxygen affinity of an iron(II) bis(pocket porphyrin), which provides nonpolar surroundings to the binding site, was reported to be sensitive to small variations in the polarity of aprotic-substituted benzene solvents.[5] Dioxygen affinity was higher in solvents of higher polarity, while the converse was true for the binding of carbon monoxide.[5]

The above findings have led us to take a closer look at the data from X-ray single crystal investigations of derivatives of the iron(II) "pickel-fence" porphyrin Fe(TpivPP) and to attempt to quantify the distal O_2···picket interactions.[16] Some of the relevant distances and angles are summarized in Figure 1. The shorter N(H) ···O separations of 3.88 and 4.19 Å can be compared with typical values of 2.94 Å for associative hydrogen bonding in primary amides[17] and 2.85 Å for secondary amides[18] where strong

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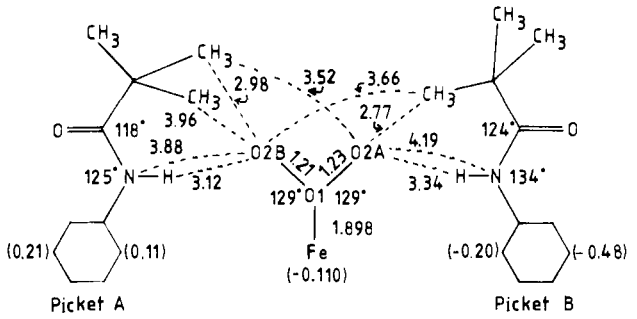

Figure 1. Selected metrical details of the O_2···pivalamide interactions. For atom labeling conventions and full metrical details see ref 16. The separations O2A···N(picket A) and O2B···N(picket B) are 4.85 and 5.06 Å and 5.22 and 5.19 Å, respectively. The numbers in brackets are displacements in Å from the plane of the 24-atom porphyrinato skeleton.

hydrogen-bonding interaction exists. The separations here are ~1 Å longer, and further contributing to the weakness of this hydrogen bond is the nonlinear arrangement of N–H···O atoms. As we will show, this corresponds to a significant although weak hydrogen-bonding interaction.[19,20] Moreover, only a 2.8 kcal/mol change in free energy is needed to account for a 2 orders of magnitude change in an equilibrium constant at room temperature. Finally we should point out that the system is geometrically constrained to have the terminal oxygen atom and the N–H group pointing toward each other so that the entropy associated with the work of bringing two particles together is not relevant.

Recently it was suggested, based on intramolecular ligand–picket distances for Fe(TpivPP)(2-MeIm)(O_2) and Fe(TpivPP)(1-MeIm)(O_2) complexes that there exist substantial repulsive steric interactions between the methyl groups and the dioxygen ligand. On the other hand, for the CO derivative, no such clash occurs.[3] It is of interest to gain some appreciation of the relative magnitude of the various interations that an O_2 or CO molecule undergoes with the pocket. It should be emphasized that we are not attempting to calculate absolute values for these interactions. The results are expected to have significance when relative values are considered.

Methods

In order to make such calculations, an estimate is needed for the charge on the O_2. Experimental and theoretical results on Co–O_2 species show that the extent of charge transfer onto O_2 varies from –0.1 to –0.9 e^- depending on the field strength of the other ligands, especially the axial base.[6,21] Most calculations on comparable iron systems show a few tenths of an electron less, with values in the range 0 to –0.75 e^-.[22-24] Thus, we have selected for Fe–O_2 systems a value of –0.25 e^- and distributed this over the two oxygen atoms as –0.1 e^- on the bound dioxygen, O1, and –0.15 e^- on the terminal oxygen atom, O2. The ratio is similar to that calculated recently, although the total extent is about one-half.[24] For Co–O_2 systems, we have chosen values of Q_{O1} = –0.2 e^- and Q_{O2} = –0.3 e^-,[33] giving a total of –0.5 e^-, in accord with values derived from EPR data.[6]

Table I. Potential Parameters for O_2···Pivalamide and CO···Pivalamide Contacts[a-c]

atom type	A, kcal mol^{-1} Å^{12}	B, kcal mol^{-1} Å^6	Q
N(H)	2271 × 10^3	1230	–0.28
H(N)	0	0	0.28
O(RCO)	275 × 10^3	502	–0.38
C(RCO)	3022 × 10^3	1340	0.28
H(HCO)	7150	32.9	0.10
C(CCO)	1811 × 10^3	532	0.10
C(CH_3)	1811 × 10^3	532	–0.30
H(CH_3)	7150	32.9	0.10
O(bonded)[d]	275 × 10^3	502	–0.10
O(terminal)[d]	275 × 10^3	502	–0.15
C(CO)	3022 × 10^3	1340	0.05
O(CO)	275 × 10^3	502	–0.05

[a] Values for the amide moiety are taken from ref 25. The fragments CH_3, NH, and RCO where R=H or the quaternary carbon atom have 0 net charge. [b] The dioxygen atoms are assumed to resemble amide oxygen atoms. At the distances involved for the O_2...amide contacts, the values of A and B are unimportant and similarly for the carbonyl ligand for which parameters from the amide carbonyl moiety have been appropriated. [c] Rather similar values have been produced by a wide variety of theoretical calculations. For a summary see ref 25a. [d] These values pertain to an FeO_2 adduct. For the corresponding CoO_2 adduct $Q_{O(bonded)}$ = –0.20 e^- and $Q_{O(terminal)}$ = –0.30 e^-.

Using a Lennard-Jones potential function with an additional term for electrostatic contributions, the interaction energy (ΔE) between two molecular fragments is given by

$$\Delta E = \sum_{i,j>i} \left(\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{Q_i Q_j}{R_{ij}} e_0^2 \right)$$

where the sum is over all pairs of interactions between, in this case, the dioxygen and pivalamide (picket) components, e_0^2 = 332 Å kcal/mol, Q_i and Q_j are the fractional charges, $A_{ij} = (A_i A_j)^{1/2}$, and $B_{ij} = (B_i B_j)^{1/2}$.

(19) A hydrogen bond, X-H···Y, is generally considered to exist if the sum of the van der Waals radii $W_H + W_Y > d_{H\cdots Y}$. Even for strong unsymmetrical hydrogen bonds, the extent of electron transfer from Y onto H is very small (0.04 e^-).[20] Viewing hydrogen bonds as primarily electrostatic interactions that decrease slowly as $1/d$ leads to a more relaxed definition: a hydrogen bond exists if a hydrogen atom is positioned between two electronegative atoms in an approximately linear fashion.

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(22) (a) Dedieu, A.; Rohmer, M. M.; Veillard, A. In "Metal-Ligand Interactions in Organic Chemistry and Biochemistry"; Pullman, B., Goldblum, N., Eds.; Reidel: New York, 1977; Part 2, pp 101–130. (b) Dedieu, A.; Rohmer, M. M.; Benard, M.; Veillard, A. *J. Am. Chem. Soc.* **1976**, *98*, 3717–3718. These ab initio calculations which predicted essentially neutral oxygen atoms also predicted that a putative NH_3···O_2 interaction would be repulsive. Experimental evidence is now clearly to the contrary.[5-15]

(23) Case, D. A.; Huynh, B. H.; Karplus, M. *J. Am. Chem. Soc.* **1979**, *101*, 4433–4453 and references therein.

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(27) The values for Q_i used here and taken from ref 25 agree closely with those calculated by a variety of techniques, including ab initio, CNDO, and Del Re methods. These and the coefficients A_i and B_i derived from dipole moments, heats of sublimination, and crystal packing have been used to successfully calculate crystal structures of other amides. The values in ref 26 are derived from the deformation density distribution of acetamide, and the Coulombic interaction has been separated into three components (charge, dipole, and quadrupole moments). Comparison of the Q_i here with those used[25] is therefore inappropriate.

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(29) A variety of values can be found.[39] Since experimental observations indicate little polarity in the CO moiety,[4] Q_O and Q_C have been calculated from the dipole moment of the free ligand. The A_i and B_i terms are assumed to be similar to those of a C=O group.

(30) (a) Rohmer, M.-M.; Loew, G. H. *Int. J. Quantum Chem., Quantum Biol. Symp.* **1979**, *6*, 93–104. With a thiolate axial ligand Q_C = 0.02 e^- and Q_O = –0.59 e^-; this may be compared with values of Q_{O1} = –0.34 e^- and Q_{O2} = –0.58 e^- calculated by the same method and Q_{O1} = –0.27 e^- and Q_{O2} = –0.48 e^- when the base is imidazole. (b) Values of Q_O = 0.03 e^- and Q_C = 0.21 e^- have been calculated,[2o] but parallel calculations on FeO_2 systems led to Q_{O1} = 0.14 e^- and Q_{O2} = –0.08 e^-, rather less than other calculations and inferences from experiments.

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(32) For example see the reviews: (a) Jameson, G. B.; Robinson, W. T.; Ibers, J. A. In "Hemoglobin and Oxygen Binding"; Ho, C., Ed.; Elsevier: North Holland, 1982. (b) Jones, R. D.; Summerville, D. A.; Basolo, F. *Chem. Rev.* **1979**, 139–179.

(33) Dedieu, A.; Rohmer, M.-M.; Veillard, A. *J. Am. Chem. Soc.* **1976**, *98*, 5789–5800. For a Co–O_2 Schiff base system, Q_{O1} = –0.34 e^- and Q_{O2} = –0.16 e^- were derived from population analysis. Although we have placed more density on the terminal rather than the bonded atom, the CCF calculations here, as noted before, are not very sensitive to the distribution of charge since the distances to the amide groups are rather similar for the two oxygen atoms.

Table II. Factors Influencing the O_2 Affinities of Myoglobin and "Picket-Fence" Porphyrin Complexes[a]

	MbO_2	$Fe(piv_35CIm)$[b]	Fe(TpivPP) (1-MeIm)[c]
$P_{1/2}(O_2)$, mmHg	0.7	0.58	0.5
$P_{1/2}(CO)$, mmHg	0.018	0.000 022	
solvent	H_2O/PO_4^{3-}	toluene	solid state
local O_2 environment	His (H_2O) polar, protic	H–N (amide) polar, aprotic	H–N (amide) polar, aprotic
O_2...distal group, Å	2.97 (18)	? (similar to other PF cmpd)	*N*–H, 4.06 (5); CH_3, 2.67
CO...distal group, Å	2.7	? (similar to other PF cmpd)	*N*–H, 5.0; CH_3, 3.46
changes in molecular bulk on O_2 coordn	very small	? (similar to other PF cmpd)	small decr.
Im, deg	1°	? (prob. 45°)	20°
Im, deg	0°	? (prob. 45°)	45°

[a] Abbreviations: H_2TpivPP, *meso*-α,α,α,α-tetrakis(orthopivalamidophenyl)porphyrin; 1-MeIm, 1-methylimidazole; 2-MeIm, 2-methylimidazole, H_2piv_35CIm, [*meso*-α,α,α-(orthopivalamidophenyl)porphyrinato]-β-[ortho-((4-(*N*-imidazolyl)butyl)amido)phenyl]porphyrin. [b] Reference 35. The orientation of the axial base in solution is probably 45° (for minimum steric clash with the porphyrin). The O_2 binding pocket is probably very similar to those for Fe(TpivPP)(1-MeIm)(O_2)·$^1/_2$toluene·$^1/_2$1-MeIm and Fe(TpivPP)(2-MeIm)(O_2)·EtOH with the difference that one picket bearing the axial base is oppositely directed to the other three. [c] References 16 and 28. The O_2 affinity was measured on a desolvated sample. The crystal structure was determined on the (1-MeIm)–toluene solvate. Differences in the O_2...pivalamide stereochemistry are predicted to be very small.

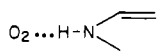
Values for the individual atom contributions A_i and B_i to the pairwise repulsive and attractive coefficients A_{ij} and B_{ij}, respectively, are given in Table I.[25] Other potential functions and Q_i are available.[26,27] Some of the R_{ij}, the internuclear separations in angstroms, are shown in Figure 1 for the complex Fe(TpivPP)(2-MeIm)(O_2)·EtOH.[16] Similar results may be expected for the related Fe(TpivPP)(1-MeIm)(O_2)·$^1/_2$(toluene)·$^1/_2$(1-MeIm) species[28] (see Table II), but since the overall precision is lower and the disorder of the *tert*-butyl groups more extensive, the corresponding calculations were not performed. We will consider only those nonbonded intramolecular contacts peculiar to the picket-fence porphyrin (compared to tetraphenylporphyrin), namely the O_2···pivalamide interaction.

Results and Discussion

Considering first only the contacts between O_2 and the four surrounding HNC(H)O moieties, we obtain a calculated energy of −9.5 kcal/mol for both orientations of the disordered dioxygen ligand. Concentrating the charge on the terminal oxygen atom alone leads to a slightly stronger attraction. For the complete pivalamide groups, the net attraction is decreased to −7.9 kcal/mol (orientation O2A) and −5.2 kcal/mol (orientation O2B), due to repulsive terms (individually as great as 3.6 kcal/mol) between the dioxygen and methyl groups. It is reassuring that the lower energy (−7.9 kcal/mol) is associated with the terminal oxygen site that has the higher relative occupancy factor (58 (3)%) in the crystal structure determination,[16] although from the Boltzmann distribution, $N_1/N_2 = \exp(-\Delta E/RT)$, this corresponds to an energy difference of only 0.19 kcal/mol. However, quite apart from inaccuracies in the CCF model itself and the imprecision in the hydrogen atom coordinates,[16] no account is made of the inequivalence of the two dioxygen sites arising from the positioning of the axial base.

The binding of CO (Q_C = 0.05 e$^-$ and Q_O = −0.05;[29,30] Fe–C 1.77 Å, CO = 1.15 Å, Fe–CO = 180° adopted from ref 31) showed only weak attractive interactions between the CO ligand and both the amide and the *tert*-butyl moieties, amounting to −1.0 and −2.8 kcal/mol, respectively.

Table II shows the interplay of factors which lead to the similarities and differences in the binding of O_2 and CO to myoglobin and to the picket-fence porphyrin model. In the binding of O_2 for both species, there is an



interaction, while in Mb an eclipsing conformation of the dioxygen ligand enhancing repulsive N(porph)···O interactions is paralleled in the model system by O_2···CH_3 repulsions. On the other hand, for the model system, the CO ligand nestles comfortably in the binding pocket, whereas for Mb close repulsive CO···N interactions are unavoidable.

Although the structure of a Co(TpivPP)(2-MeIm)(O_2)·EtOH complex remains undetermined, it can be predicted with confidence that it will be closely isostructural to within 0.1 Å of the iron analogue, with a Co–O separation of 1.95 Å by comparison with related structures.[32] With the charge distributed as Q_{O1} = −0.20 and Q_{O2} = −0.30 e$^-$[33] interaction energies of −18.0 and −18.1 kcal/mol with the amide groups and −16.4 and −13.7 kcal/mol with the complete pivalamide groups are calculated. Not surprisingly the greater charge on the dioxygen leads to a stronger attraction. Recently an *N* parameter, a relative discrimination factor for dioxygen binding to cobalt compared to the corresponding iron porphyrinato system, was defined[3]

$$N = \frac{P_{1/2}(O_2)\text{–Co}}{P_{1/2}(O_2)\text{–Fe}}$$

For the picket-fence porphyrin and myoglobin, this number was noticeably smaller than those for other porphyrins. This observation is consistent with enhanced O_2 binding to Co species relative to Fe species as a result of the greater stabilization possible if electrostatic interactions exist between polar distal groups, H–X, and the more polarized CoO_2 moiety. A decrease in the differential stabilization results.

In the NMR study of O_2 binding to a iron(II) tetrakis(orthoamidophenyl)porphyrinato species, an O_2···amide separation of 3.0 Å was deduced.[7] At such separations, the O_2···amide attraction increases to −14 kcal/mol for an Fe–O_2 complex and to −27 kcal/mol for an analogous Co–O_2 adduct. It is conceivable, therefore, that under certain stereochemical constraints, the energy to be gained from a strong hydrogen bond may counterbalance the energy needed to linearize the M–O–O bond. For a Co–O_2 species, the energy calculated to change this angle from 120° to 155° is 15 kcal/mol.[33] In ErO_2 and $Co(CN)_5(O_2)^{3-}$, the dioxygen is hydrogen bonded to H_2O, and M–O–O angles greater than 150° are observed, although in the latter it has been postulated that the adjacent CN^- ligands force a partial linearization.[34]

Furthermore, for a hemoglobin with dioxygen in its preferred bent geometry and in a bisecting conformation with respect to the M–N(porph) bonds, and with a strong hydrogen bond to a distal moiety, O_2 affinities much higher than those for myoglobin can be expected. The barrier to rotation of the O_2 ligand has been calculated to be 5–6 kcal/mol,[22b] and our simple CFF model, which of course ignores bonding effects, yields a value of 2 kcal/mol. Thus, for MbO_2 relative to picket-fence porphyrin model systems, the stabilization achieved through stronger hydrogen bonding (see Table II) is counterbalanced by the eclipsed conformation adopted in the former.

Although these calculations are approximate several useful conclusions may be drawn about contacts between coordinated dioxygen and distal moieties.

(1) Electrostatic terms, both attractive and repulsive, dominate the O_2···pivalamide interactions, even for rather small amounts of charge transfer.

(2) There is a substantial net attraction between O_2 and amide groups and a smaller repulsive interaction between O_2 and the methyl groups which lowers the net attraction.

(3) The picket-fence porphyrin offers a binding pocket for CO where weak, still predominantly electrostatic, forces yield a small net CO···pivalamide attraction.

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Table III. Dioxygen and Carbon Monoxide Affinities (mmHg) of Selected Hemoglobins

hemoglobin	ref	$P_{1/2}(O_2)$	$P_{1/2}(CO)$	temp, °C
Hb ascaris	36	$1-4 \times 10^{-3}$	1.0×10^{-1}	20
	37[a]	4.7×10^{-3}	6.3×10^{-2}	27.5
leg Hb	38[b]	4.7×10^{-2}	7.4×10^{-4}	20
	38[c]	4.5×10^{-2}	7.1×10^{-4}	25
horse Mb	39	7.0×10^{-1}	1.8×10^{-2}	20
Hb aphrodite	40[d]	1.1	6.5×10^{-3}	20
Hb^{A}R	35a[e]	0.15-1.5	$1-4 \times 10^{-3}$	
Hb^{a}T	35a[e]	9-160	$1-2.8 \times 10^{-1}$	

[a] These values are obtained from the ratios for the rate of binding and of dissociation, k_{on}/k_{off}, and converted by using the following values for the solubility of O_2 and CO in water; 1.82×10^{-6} and 1.36×10^{-6} M/torr, respectively.[41] [b] The value for $P_{1/2}(O_2)$ was calculated from $P_{1/2}(CO)$ and the partition coefficient, $M = 64$. A value of 4×10^{-2} torr was measured directly.[42] [c] Values are obtained from the ratio of k_{on}/k_{off}. See footnote a for conversion. [d] $P_{1/2}(CO)$ is calculated from $P_{1/2}(O_2)/M$, where $M = 167$. [e] The values reported therein are a compilation from the original literature and are sensitive to pH, ionic strength, phosphate concentration, etc. The temperatures are in the range 15-25 °C.

(4) Even with small amounts of charge transfer onto the O_2 ligand, a substantial attraction exists at distances much longer than conventionally taken as indicative of strong hydrogen bonds. Thus, hydrogen bonding and the absence or minimization of repulsive contacts with other groups, such as the porphyrin, may be a major factor in the means by which hemoglobin ascaris, for example, achieves a dioxygen affinity nearly 3 orders of magnitude greater than vertebrate hemoglobins, such as myoglobin (see Table III).

(5) In the maximization of electrostatic attractions between coordinated dioxygen and an H-X moiety, where X is an electronegative atom or group, substantial distortions (up to 25°) of M-O-O bond angles from expected angles of 120° may result.

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Registry No. O_2, 7782-44-7; CO, 630-08-0; pivalamide, 754-10-9.

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Flavin Analogue-Metal Ion Complexes Acting as Efficient Photocatalysts in the Oxidation of *p*-Methylbenzyl Alcohol by Oxygen under Irradiation with Visible Light

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Abstract: Flavin analogues (3-methyl-10-phenylisoalloxazine and 3-methyl-10-phenyl-5-deazaisoalloxazines) have been found to catalyze the photooxidation of *p*-methylbenzyl alcohol by oxygen efficiently in the presence of Mg^{2+} or Zn^{2+} ion in acetonitrile to yield *p*-methylbenzaldehyde and hydrogen peroxide under irradiation of the visible light of $\lambda > 360$ nm. The Mg^{2+} or Zn^{2+} ion forms a 1:1 complex with flavin analogues in acetonitrile. Such a complex formation between flavin analogues and the metal ion not only increases the oxidizing ability of the excited states of flavin analogues as indicated by the significant acceleration in the fluorescence quenching rate of flavin analogues with electron donors (methyl- and methoxy-substituted benzenes) by the complex formation but also stabilizes flavin analogues against irradiation with visible light to prevent the photodegradation of flavin analogues. The photocatalytic oxidation of *p*-methylbenzyl alcohol by oxygen is shown to proceed mainly via the singlet-excited states of flavin analogue-metal ion complexes acting as efficient photocatalysts.

Photochemistry of flavin and its analogues has been the subject of intense research, since photochemical activation of flavin analogues makes it possible to oxidize substrates which cannot be oxidized thermally.[1-6] As such, photoreduction of flavin analogues by a variety of substrates such as amino acids, amines, carboxylic acids, and NADH[1-5] or intramolecularly by the ribityl

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