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Iron(III) complexes of phenolate ligands as models for catechol dioxygenases

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Abstract. Catechol 1,2-dioxygenase (CTD) and protocatechuate 3,4-dioxygenase (PCD) enzymes catalyse the oxidative cleavage of catechols to *cis, cis*-muconic acids with the incorporation of molecular oxygen. In our laboratory two series of iron(III) complexes of linear tridentate and tripodal tetradentate phenolate ligands have been characterised using IR, UV-Vis and EPR spectral and electrochemical techniques. The X-ray crystal structure of a few of the complexes have been determined. The interactions of the complexes with a variety of monodentate and bidentate heterocyclic bases as well as phenols have been investigated. The interactions with catecholate anions reveal changes in the phenolate-to-iron(III) charge transfer band, which are remarkably similar to catechol dioxygenase-substrate complexes. The redox behaviour of the complexes and their 1:1 adducts with 3,5-di-*t*-butylcatechol (H_2DBC) has been investigated. All the complexes catalyse the oxidative cleavage of H_2DBC by molecular oxygen to yield *cis,cis*-muconic anhydride. The structure, redox and catalytic activities of the iron(III) complexes have been discussed *vis-a-vis* those of the enzymes.

Keywords. Iron(III) complexes; X-ray structure; electronic spectra; electron paramagnetic resonance spectra; redox behaviour; dioxygenase activity.

1. Introduction

Catechol 1,2-dioxygenase (CTD) and protocatechuate 3,4-dioxygenase (PCD) are bacterial non-heme iron enzymes (Lipscomb and Orville 1993). They catalyze the oxidative cleavage of catechols to *cis,cis*-muconic acids with the incorporation of molecular oxygen via a mechanism involving a high-spin ferric centre (Que 1980, 1983). Based on various spectroscopic studies, the active sites of these enzymes have been proposed (Que *et al* 1976, 1980; Que and Epstein 1981; Felton *et al* 1982; Whittaker and Lipscomb 1984; Whittaker *et al* 1984; Kent *et al* 1987) to consist of a ferric centre co-ordinated to two tyrosines, two histidines, and a water molecule. In fact the X-ray crystal structure (Ohlendorf *et al* 1988) of PCD has revealed that the active site iron(III) is liganded by four residues, viz. Tyr-108, Tyr-147, His-160 and His-162 and a solvent molecule to form an approximate trigonal bipyramidal geometry. Several iron(III) complexes have been synthesised to mimic the iron(III) coordination site and substrate interaction of the enzymes as well as the chemistry of the catalytic cycle. However, models which mimic both the catalytic activity and spectral behaviour of the iron site in these enzymes are scarce (Nishida *et al* 1984). Thus, earlier iron(III) complexes (Que

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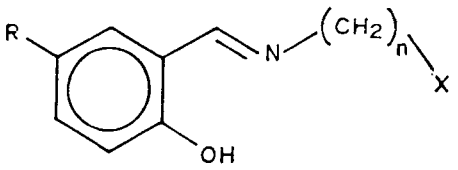
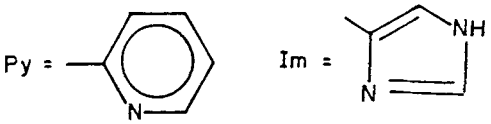
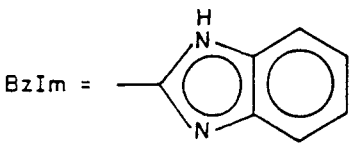
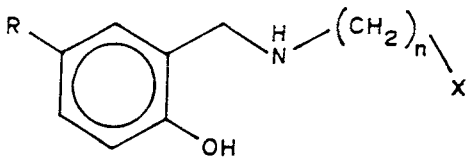


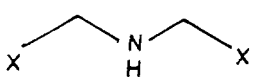
Ligand	R	n	X
L1	H	1	Py
L4	H	2	Im
L5	H	1	BzIm



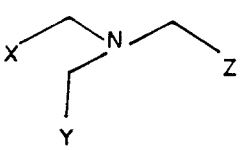


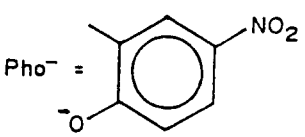


Ligand	R	n	X
L2	$-NO_2$	1	Py
L3	$-NO_2$	2	Py
L6	H	1	BzIm
L7	NO_2	1	BzIm



L8	X = BzIm
L9	X = Py





Ligand	X	Y	Z
L10	Py	Py	Py
L11	Py	Py	Pho^-
L12	Py	Pho^-	Pho^-
L13	BzIm	BzIm	BzIm
L14	BzIm	BzIm	Pho^-
L15	BzIm	OH	Pho^-

Scheme 1. Ligands employed in the study.

and Heistand 1979; Heistand *et al* 1982) of salen [salen = N,N′-ethylene*bis*-(salicylaldimine)] successfully mimicked many of the spectroscopic properties but not the catalytic activity of the enzyme. Que and coworkers synthesised and studied a series of

[Fe(L)DBC] complexes (Que *et al* 1987; Cox and Que 1988; Cox *et al* 1988), where L is a tetradentate tripodal ligand like TPA [TPA = *tris*(pyrid-2-yl- methyl)amine] or NTA (NTA = nitrilotriacetic acid) or a NTA analogue with one or two carboxylate pendants and H_2DBC is 3,5-di-*t*-butylcatechol.

Only two of the tripodal ligand complexes (L11, scheme 1), recently reported (Nishida *et al* 1984; Cox and Que 1988; Cox *et al* 1988) to effect oxidative cleavage, contain a coordinated phenolate and no tripodal analogue reported so far contains one/two phenolic hydroxyl groups as well as imidazole nitrogen donor(s) though dioxygenases contain two tyrosine and two imidazole functions coordinated to iron(III). Thus clearly no model reported so far possesses all the features of the dioxygenase active sites. The present paper describes our successful attempt to synthesise and study Fe(III) complexes of systematically varied tri-(Viswanathan and Palaniandavar 1995) and tetradentate tripodal ligands (Viswanathan *et al* 1996) containing one or two phenolate functions along with benzimidazole (bzim) or pyridine (py) moieties (scheme 1), to closely mimic the enzyme active site. The tripodal ligand systems designed for the present study are expected to generate the non-heme iron environment (Ohlendorf *et al* 1988) of the enzymes and encourage asymmetric coordination proposed (Whittaker and Lipscomb 1984) to be essential for the cleavage of H_2DBC. Our effort is directed towards investigating the phenolate-to-Fe(III) CT spectra, redox and reactivity of the complexes. Our study is expected to afford improved understanding of the structure-spectra and structure-function correlations for the active site of the enzymes, verify the validity of the proposed oxidative cleavage mechanism for phenolate complexes and thereby provide an assessment of the importance of tyrosinase coordination in the enzyme.

2. Experimental

The synthesis and characterisation of the present iron(III) complexes and the experimental methods employed for studying them have been described previously (Viswanathan and Palaniandavar 1995; Viswanathan *et al* 1996).

3. Results and discussion

Based on the results of elemental analysis the formulae of the Fe(III) complexes have been assigned. The present ligands provide a reasonable analogue to histidine and tyrosinate coordination in CTD enzyme via the bzim and phenolate moieties. Further, the tridentate ligands are varied by the saturation of the imine function to disrupt the conjugation between the functionalities and to allow more flexibility in chelation to the metal ion. The tetradentate tripodal ligands based on trimethyl amine (scheme 1) with the pendant functionalities varying from py to bzim to phenolate have been also synthesised to provide a systematic variation of the ligand Lewis basicity and charge while retaining similar coordination geometries in ternary iron(III) complexes with catechols. The bulky bzim ring(s) seems to be a good choice to offer steric hindrance to the added ligands or substrates as in proteins so as to closely approximate the active site in enzymes.

3.1 *Structures of iron complexes*

All the iron(III) complexes have magnetic moments in the range 5·8–5·9 BM at room temperaure, consistent with a high-spin ferric centre with five unpaired electrons. In the

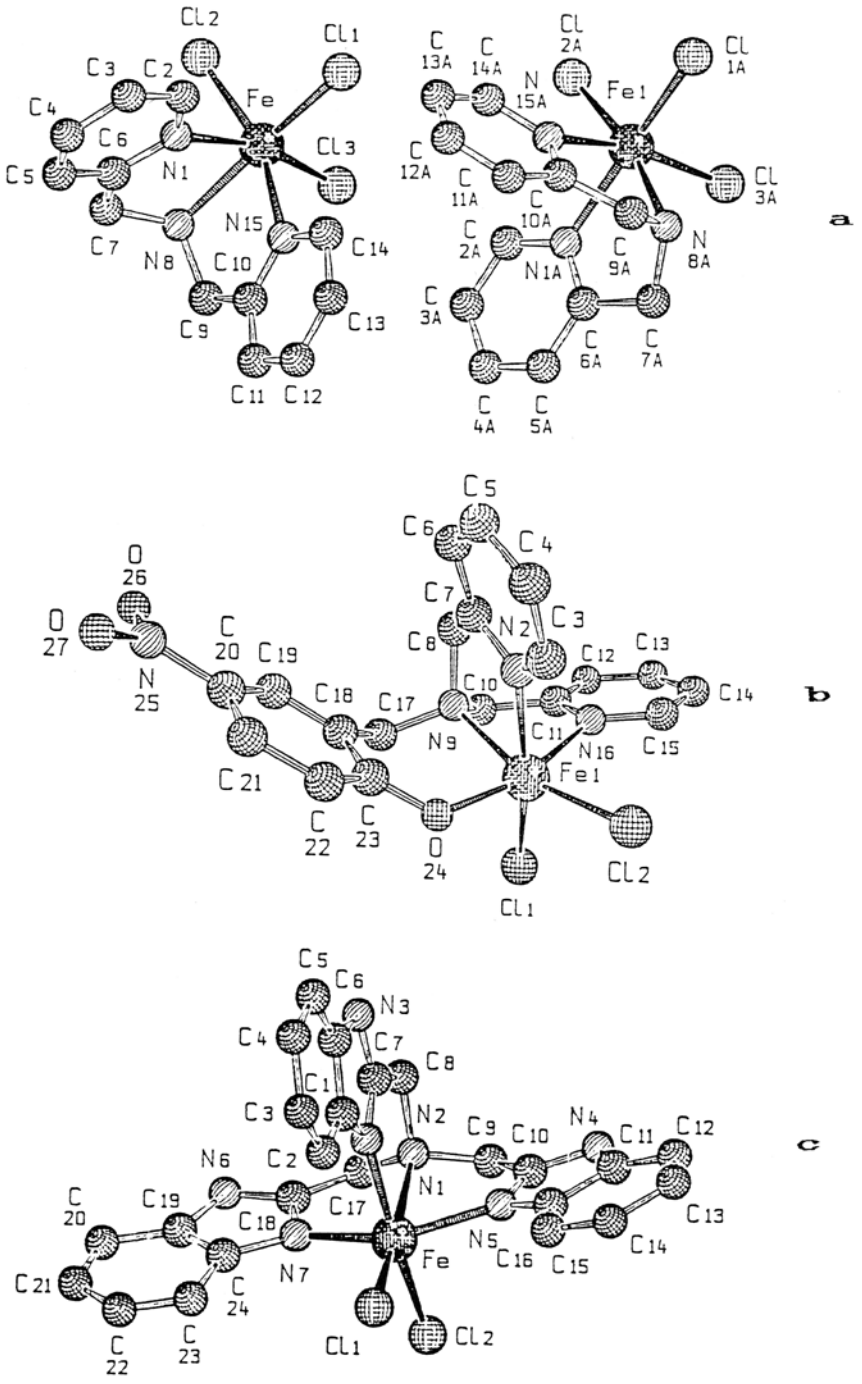


Figure 1. X-ray crystal structure of $Fe(L9)Cl_3$ (**a**), $Fe(L11)Cl_2$ (**b**) and $Fe(L13)Cl_3$ (**c**). $Fe(L9)Cl_3$ contains two crystallographically independent molecules present in the asymmetric unit.

X-ray crystal structures of $Fe(L16)Cl_3$ (Adams *et al* 1990) [L16 = *bis*(benzimidazol-2-ylmethyl)methylamine] and $Fe(L9)Cl_3$ (Viswanathan *et al* 1996) [L9 = dipicolylamine] (figure 1), the homologue and pyridine analogue respectively of $Fe(L8)Cl_3$, all the three chloride ions are coordinated. In the crystal structures of $Fe(L11)Cl_3$ and $[Fe(L13)Cl_2]Cl$ (Viswanathan *et al* 1996), iron(III) is octahedrally co-ordinated (figure 1). Spectral studies suggest similar structures for the other Fe(III) complexes. The chloride ions are replaced by solvent molecules or polar groups of the neighbouring ligand molecules in solution, as revealed by conductometric studies.

As evident from the above crystal structures, two *cis* coordination sites are open in all the present complexes for the coordination of two monodentate or one bidentate base molecule like H_2DBC and the complexes are convenient for the investigation of the effect of adduct formation on the spectra, In fact, the hyperfine broadening observed from O^{17}-enriched water for the 4-HBA-PCD complex (Whittaker and Lipscomb 1984; Orville and Lipscomb 1989) (4-HBA = 4-hydroxybenzoate) has shown that at least two coordination sites of Fe(III) appear adjacent and accessible to exogenous ligands.

3.2 *Electronic absorption spectra*

The electronic spectra of all the present iron(III) phenolate complexes in methanol solution (table 1) exhibit two bands in the near UV (≈ 410 *sh*, ≈ 330 nm) and one in the visible (550–470 nm) regions. The visible band which is absent in complexes of L8, L10 and L13, which lack a phenolate function may be assigned (Cassella *et al* 1987) to phenolate $(\pi_1) \rightarrow Fe^{III}$ $[d_\pi(d_{xz})]$ charge transfer transition. The 400 nm band observed for all the complexes may originate (Cassella *et al* 1987) from the filled *d*-orbitals of iron(III) to the antibonding orbitals of the phenolic residue. The conjugated imine $\pi \rightarrow \pi^*$ transition in the salicylaldimine residue is observed as an intense band around 330 nm.

The position and intensity of the visible band shows a remarkable dependence on the nature of the ligand. The increasing orders of its energy: Fe(L4) < Fe(L5) < Fe(L1) < Fe(L3) < Fe(L6) < Fe(L7) < Fe(L2); Fe(L11) < Fe(L12) ≤ Fe(L14) < Fe (L15) represent the decreasing order of Lewis acidity of the ferric centre. The iron *d*-orbital energies are raised (Que *et al* 1987) by the negative charge built on Fe(III) by the higher basicity of imine compared to –NH– nitrogen and of im compared to bzim, and by the replacement of py/bzim by the second phenolate group. Further, the electron withdrawing NO_2 group would be expected to decrease the band energy but an increase is observed for $Fe(L2)Cl_2$ as compared to $Fe(L1)Cl_2$. MOPAC calculations reveal (Viswanathan and Palaniandavar 1995) that the observed blue shift is due to lowering in energy of the ligand orbitals on the introduction of *p*-NO_2 group.

Further, the absorptivities of the visible bands of the present complexes vary widely and do not show any regular trend. All these observations show that band positions as well as intensities are very sensitive to the iron environment; this is reminiscent of native CTD and PCD enzymes (Stewart 1989).

3.3 *Adduct formation*

On addition of secondary ligand molecules the position of the visible band shifts generally to higher energy with modest changes in intensity. For tridentate ligand complexes addition of N-methylimidazole (mim) shifts the visible band to higher energies with decrease in absorptivity for almost all the complexes. A similar but lesser

Table 1. $PhO^- \rightarrow Fe(III)$ charge transfer spectral [a] and redox potential (volts) data for Fe(III) tri- and tetradentate tripodal ligand complexes.

	$\lambda_{max}(\varepsilon_{max})$		$E_{1/2}$(V)		
Complex	Complex	DBC adduct	Fe(III)/Fe(II)	$DBSQ^-/H_2DBC$	Yield
$Fe(L1)Cl_2 \cdot H_2O$	543(400)	640(100) 528*sh*(200)	−0·169	−0·116	19·8
$Fe(L1)Cl_2 \cdot H_2O$	478(450)	693(260) 415*sh*(1430)	−0·330	−0·244	42·4
$Fe(L3)Cl_2 \cdot H_2O$	510(300)	—	−0·260	−0·277	—
$Fe(L4)Cl_2 \cdot H_2O$	580(1730)	—	0·131	0·139	—
$Fe(L5)Cl_2 \cdot H_2O$	554(1630)	567(3990)	−0·177	−0·121	37·0
$Fe(L6)Cl_2 \cdot H_2O$	508(1540)	575(1050)	−0·887	−0·505	24·8
$Fe(L7)Cl_2 \cdot H_2O$	503(1760)	650*sh*(1710)	−0·319	−0·246	23·5
$Fe(L8)Cl_3$	436*sh*(540)	637(440) 450(1210)	0·101	0·066	13·6
$Fe(L10)Cl_3$	—	578[b] 888	0·078	—	41·0
$Fe(L11)Cl_2$	550(1650)	790(490) 490*sh*(670)	−0·101	0·008	12·0
$Fe(L12)Cl \cdot H_2O$	493(630)	850–700[c] 400*sh*(470)	−0·301	−0·210	56·0
$Fe(L13)Cl_3$	—	548[b] 842	−0·002	−0·054	18·0
$Fe(L14)Cl_2$	500(880)	768(360) 492(510)	−0·130	−0·130	59·0
$Fe(L15)Cl \cdot H_2O$	492(3120)	600–800[c]	−0·301	−0·240	50·0

[a]Viswanathan and Palaniandavar (1995); Viswanathan *et al* (1996)
[b]Cox *et al* (1988)
[c]very broad band

shift is observed for bzim; this is expected as bzim is less basic and bulkier than mim. The addition of py also decreases the λ_{max} but enhances the absorptivity of compounds of bzim-based ligands. There is a blue shift with moderate to tremendous enhancement in absorptivity for the addition of bidentate heterocyclic bases (figure 2), as expected; however, for 2,9-dimethyl-1,10-phenanthroline there is only a slight enhancement, possibly because of steric hindrance from its methyl groups. The binding of phenolate (phO^-) effects a large decrease in λ_{max} with increased or decreased absorptivity and for monoanionic $HDBC^-$ a visible band around 650 nm with very high intensity corresponding to $HDBC^- \rightarrow Fe(III)$ ($d\pi$) CT is observed. In contrast, for catecholate ($HCAT^-$) no such band is discernible and the ligand to metal charge transfer band expected for $HCAT^- \rightarrow Fe(III)$ interaction may overlap with the visible band of the original complexes. The effect on the visible band of adding dianionic CAT^{2-} is the same as that for $CATH^-$ but the intensity is enhanced further. In contrast, the effect of addition of dianionic DBC^{2-} is to increase the λ_{max} appreciably with a decrease in intensity. Further, on the addition of even neutral H_2DBC (figure 2) rather than H_2CAT the charge transfer bands are exhibited, of course, with relatively low absorptivity. The absorptivity is enhanced on the addition of Et_3N due to the deprotonation of H_2DBC. This reveals the spontaneous deprotonation of H_2DBC rather than H_2CAT, to bind strongly to Lewis acidic Fe(III).

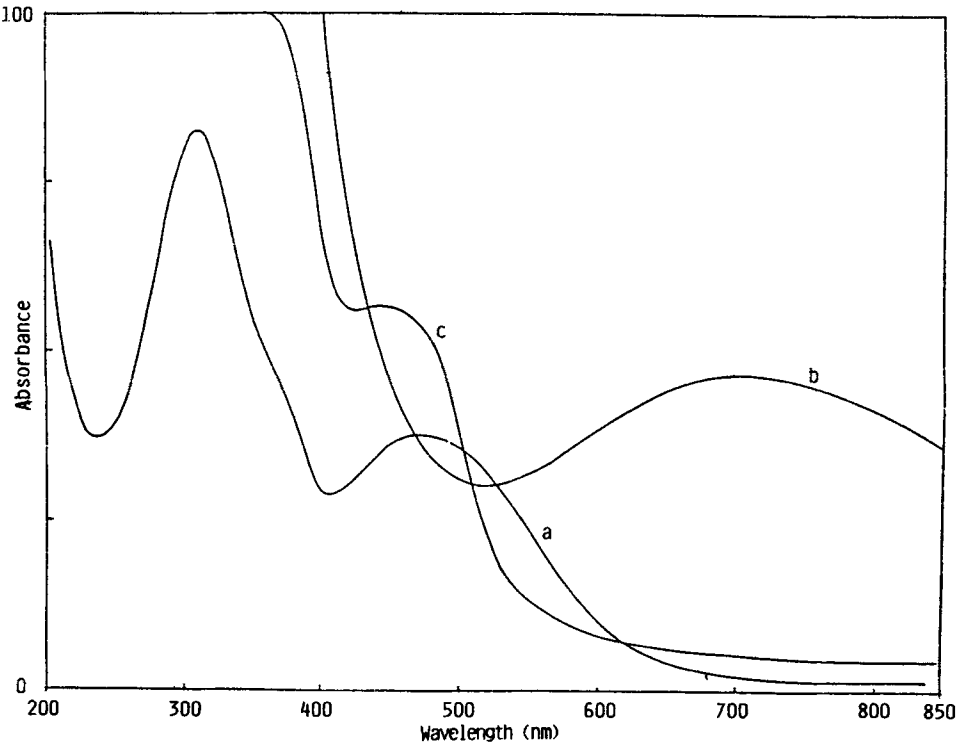


Figure 2. Electronic absorption spectra of $[Fe(L1)Cl_2]$ without (a), and with the addition of H_2DBC (b) and 1,10-phenanthroline (c).

The appearance of two new bands (430–470, 630–890 nm) on the addition of CAT^{2-}/DBC^{2-} to all the tripodal iron(III) complexes is evident from the difference spectra vs the original complex, rather than the absorbance spectra (vs methanol) of the cat^{2-}/DBC^{2-} adducts (table 1). The high energy band may correspond (Cox *et al* 1988) to the endogenous phenolate → Fe(III) charge transfer band which, as expected, has been blue-shifted on adduct formation. The low energy band may originate (Que and Heistand 1979) from CAT^{2-}/DBC^{2-} → Fe(III) charge transfer transition. Obviously, the high energy CAT^{2-}/DBC^{2-} → Fe(III) charge transfer band would have merged with the endogeneous phenolate → Fe(III) charge transfer band.

The low rather than high energy CAT^{2-}/DBC^{2-} → Fe(III) CT band exhibits remarkable dependence on the nature of the ligand and, in fact, the Lewis acidity of the iron centre, as shown by the magnitude of this band energy, decreases in the order: Fe(L2) > Fe(L7) > Fe(L1) ≥ Fe(L8) > Fe(L6) > Fe(L5); Fe(L10) > Fe(L11) > Fe(L12) and Fe(L13) > Fe(L14) > Fe(L15) respectively; the band shifts to higher energy as the softer nitrogen ligand is replaced by pendant phenolate. This also represents the decreased semiquinone character as one progresses from three py/bzim pendants to two phenolates, due to enhanced covalency of the metal-chelate interaction and lends credence to the proposed mechanism of dioxygenase activity (figure 2) (Que *et al* 1977; Cox and Que 1988; Viswanathan and Palaniandavar 1995). All these observations illustrate that the iron environment undergoes significant changes on CAT^{2-}/DBC^{2-} binding and that the energies of the charge transfer band involving the

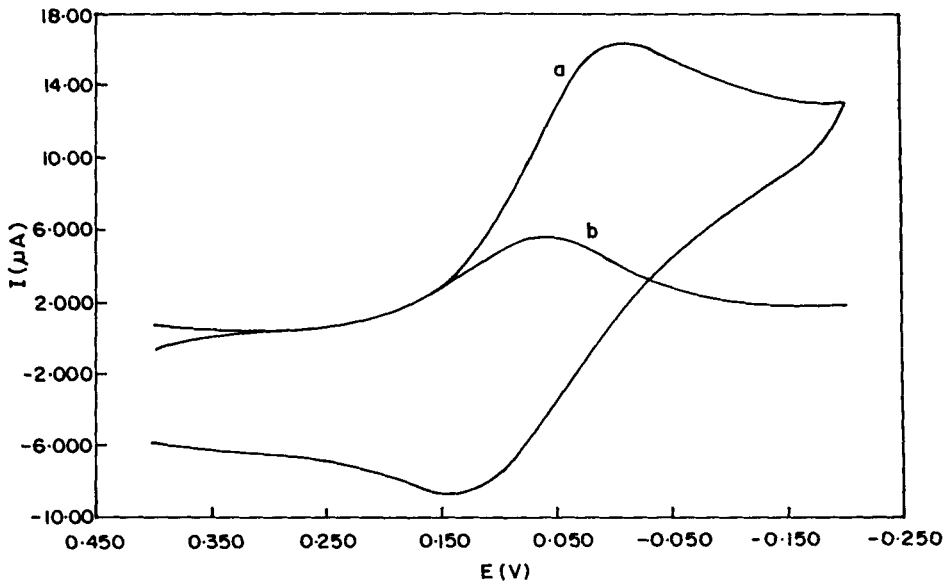


Figure 3. Cyclic (a) and differential pulse (b) voltammograms of 1 mM [Fe(L9)Cl_3] in methanol. Scan rate: CV, 0·05 V/s, DPV, 0·001 V/s and pulse height 50 mV, supporting electrolyte: 0·1 M THAP.

catecholates are modulated, interestingly, by the functionalities present in the primary ligands; the higher the Lewis basicity of the latter, the higher is the energy of the ligand to metal charge transfer bands involving the catechols.

3.4 *EPR spectra*

The powder samples of all the present Fe(III) complexes exhibit broad (100–5900 G) EPR signals near $g = 4{\cdot}3$ and $g = 2{\cdot}0$ (table 1) at room temperature, with their relative intensities varying from complex to complex. Interestingly, the complexes of L2, L10 and L12 do not exhibit the low field signal.

The well-resolved signal near $g = 4{\cdot}3$ corresponds to the one predicted for a transition between the middle Kramers doublets of rhombically distorted, high-spin monomeric iron(III) complexes. The line-width of this signal varies appreciably among the present complexes. The splitting of this signal observed for almost all the tridentate ligand complexes is similar to that observed for transferrins, a sub-class of iron(III) tyrosinate proteins (Ainscough *et al* 1980) and also for simple octahdral Fe(III) complexes (Oosterhuis 1974; Scullane *et al* 1982). The absence of such fine splitting for the tripodal complexes suggests that the ligand field is not rhombic as evident, for example, in the crystal structure of Fe(L11)Cl_2. The additional weak signal near $g = 9{\cdot}05$, observed for Fe(L5)Cl_2 may originate from a transition between ground Kramers doublets and corresponds to that observed in diferric transferrin (Aasa *et al* 1963); hence this complex may be a good synthetic model for transferrins also.

The signal found near $g = 2{\cdot}0$ in fluid solutions may arise from spin-spin coupled dimeric Fe(III) species (Borer *et al* 1983). However, the spectra of some of the complexes in fluid methanol solutions are EPR-silent, but those in acetonitrile solutions exhibit a symmetrical and relatively sharp EPR signal near $g = 2{\cdot}0$. It is

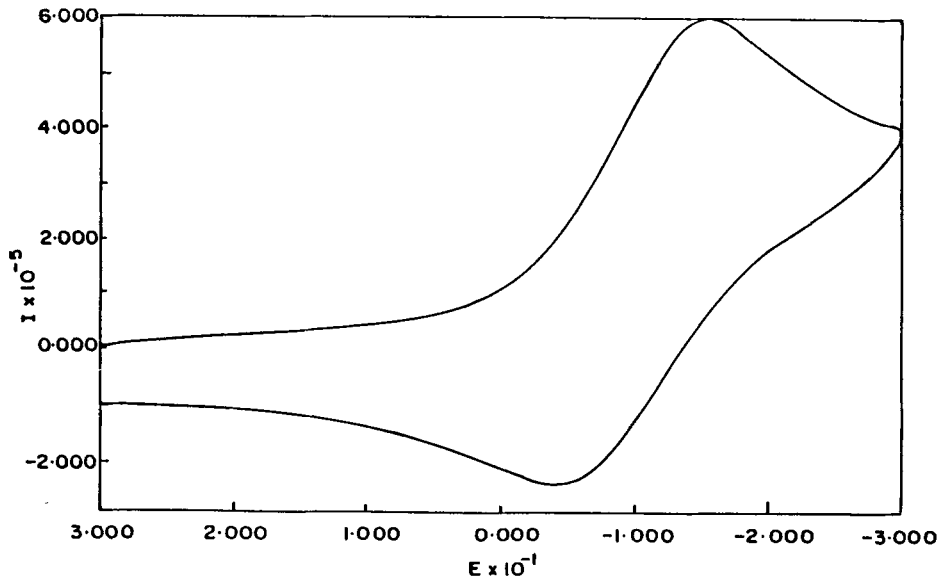


Figure 4. Cyclic voltammogram of 1 mM [Fe(L11)Cl_3] in methanol. Scan rate: 0·05 V/s, supporting electrolyte: 0·1 M THAP.

interesting to observe the disappearance of the signal when a donor base like pyridine or α,α′-bipyridyl is added, as well as its appearance on the addition of H_2DBC to the acetonitrile solution of the complexes.

3.5 *Electrochemical behaviour*

In methanol solution all the complexes exhibit fairly reversible to irreversible Fe(III) → Fe(II) redox behaviour (figures 3 and 4) (Viswanathan and Palaniandavar 1995). The value of Fe(III)/Fe(II) redox potentials follow the order: Fe(L8) > Fe(L4) > Fe(L5) > Fe(L1) ≈ Fe(L3) > Fe(L7) > Fe(L2) reflecting the decrease in Lewis acidity of the iron(III) centre by the coordination of Lewis basic phenolate and other groups with varying Lewis basicity: Fe(L10) > Fe(L11) > Fe(L12) ≈ Fe(L13) > Fe(L14) > Fe(L15), reflecting the decreasing Lewis acidity of iron(III) centre as the charge on the tetradentate tripodal ligand set increases. This trend in Lewis acidity follows that observed for the phenolate-to-iron(III) charge transfer band energy and thus the present complexes exhibit a linear correlation (Ramesh and Mukherjee 1992) between the latter and the redox potential (figure 5). This trend is consistent with that derived from CAT^{2-}/DBC^{2-} → Fe(III) and PhO^- → Fe(III) LMCT band energies.

3.6 *Electrochemical behaviour of* [*FeL(DBC)*]$^{n-}$

The adducts $[Fe(L)(DBC)]^{n-}$ were generated *in situ* in methanol solution for electrochemical investigation and attempts to isolate them were not fruitful. The cyclic and differential pulse voltammetric results corresponding to complete formation of $[Fe(L)(DBC)]^{n-}$ in which DBC^{2-} functions as a bidentate ligand, as evidenced by spectral measurements reveal a new reversible to irreversible wave corresponding to a DBSQ/DBC couple of co-ordinated (Johnson *et al* 1984; Ramesh and Mukherjee

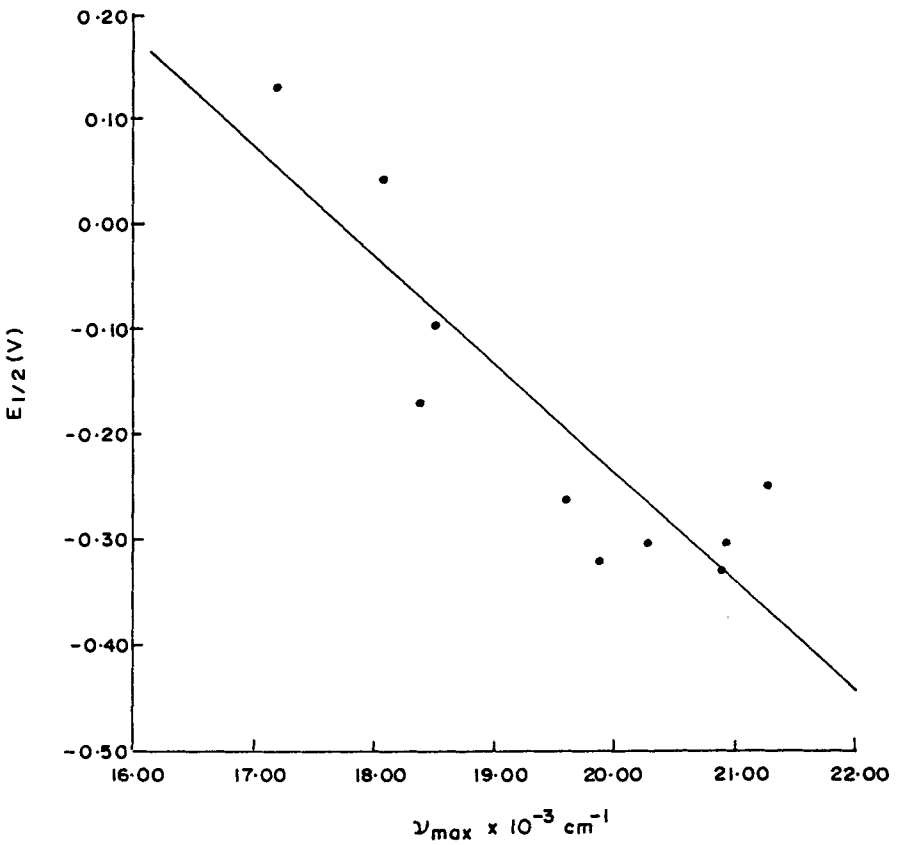


Figure 5. Linear correlation of $E_{1/2}$ of Fe(III)/Fe(II) couple with phenolate-to-iron(III) CT band energy for the present iron(III) complexes.

1992) DBC^{2-}. The irreversible reduction peak located at potentials more negative than this wave is associated with Fe(III) → Fe(II) process in $[Fe(L)(DBC)]^{n-}$. The potential of the irreversible DBSQ/DBQ couple is almost unaffected.

The redox potential of coordinated DBSQ/DBC couple (0·008 – – 0·615 V, NHE) (table 1) is considerably more positive than that of free DBSQ/DBC couple (Nanni *et al* 1980) (– 1·096 V, NHE) reflecting the significant stabilisation of DBC towards oxidation, by coordination to Fe(III) as well as the strong affinity (Avdeef *et al* 1978) of catecholates to Fe(III), even in the absence of Et_3N. Further, these potentials are comparatively more positive than, and exhibit the same trend as, that of the Fe(III)/Fe(II) couple of the parent complexes. Thus the stabilisation of the DBC oxidation state in substrate-coordinated complex is dictated by the nature of the ligand; the Lewis basic NH and phenolate donors lower the DBSQ/DBC potential, while π-delocalising by NO_2 and replacement of bzim by more π-delocalising py moiety raise it. In other words, a more Lewis acidic iron centre further stabilises the DBC oxidation state. The trend in these potentials follows the one in the DBC^{2-} → Fe(III) charge transfer band energies and a similar correlation has been obtained by Que *et al* (1987). These potentials are more positive (0·039–0·610 V) than

those for [Fe(salen)(DBC)]$^-$ (−0·179 V, NHE) (Lauffer *et al* 1981) and for [Fe(NTA)(DBC)]$^-$ (−0·024 V, NHE); while the former is unreactive (White *et al* 1984) the latter is reactive towards dioxygen. This suggests that all the present compounds should show cleavage activity. Further, as electron transfer from chelated catecholate complex to dioxygen (Que *et al* 1977) is increasingly thermodynamically favourable in decreasing order of $E_{1/2}$'s of DBSQ/DBC couple: Fe(L4) > Fe(L1) > Fe(L5) > Fe(L2) ≈ Fe(L7) > Fe(L6), the catalytic activity and hence the yield should decrease in this order (see below). For tripodal ligand complexes also a similar trend in the potentials is observed – decrease with increase in the number of coordinated phenolate groups and on replacing the more effectively π-back-bonding py by a bzim group. Similar variations in redox potentials have been associated with differing Lewis acidity of ferric centres in [Fe(L)DBC] complexes (Whittaker and Lipscomb 1984; Viswanathan and Palaniandavar 1995).

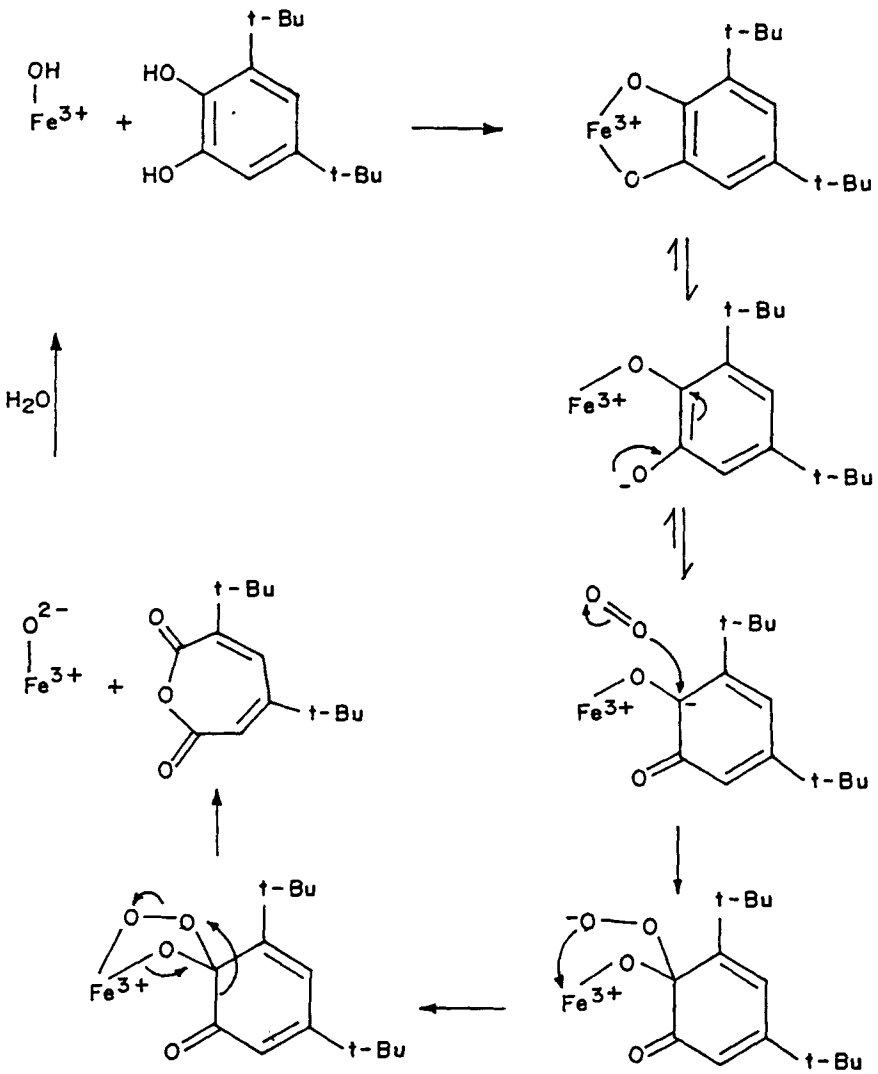
The $E_{1/2}$'s of the Fe(III)/Fe(II)-couple for almost all the DBC adducts become negative (100–500 mV) as compared to the parent complexes, suggesting (Johnson *et al* 1984) that DBC^{2-} strongly chelates to Fe(III) and that the Lewis acidity of the iron centre decreases on substrate binding. This observation is in support of the suggestion that the Fe(III) centre in the dioxygenases not only participates in the activation of the substrate but also facilitates the latter stages of the reaction. Further, the $E_{1/2}$ of the substrate coordinated Fe(III) centre reflects the reducibility of the latter to form the oxide anion (Que *et al* 1977; Ainscough *et al* 1980).

3.7 *Catalytic activity of model compounds*

The oxidative cleavage of H_2DBC was observed when it was mixed (Nishida *et al* 1984) with the present iron(III) compounds in nitromethane solution in a molar ratio of 50:1 and kept for 4 days. The complexes act as catalysts in this reaction, because the amounts of product obtained (table 1) were more than that of the complex used.

Que and coworkers proposed a novel substrate rather than oxygen activation mechanism (Que *et al* 1977; Cox and Que 1988) (scheme 2) for the dioxygenase reaction, wherein coordination to iron(III) renders the substrate susceptible to dioxygen attack because of delocalisation of unpaired spin density from iron on to catecholate. The substrate catechol loses both its protons upon coordination to the iron site and becomes susceptible to reaction with oxygen to yield a peroxide intermediate which then decomposes to the product. This mechanism implies that the yield of the desired cleavage product would increase with increase in Lewis acidity of the iron centre. Hence, the experimental yields for the present DBC adducts should follow the above experimental order of Lewis acidity. Thus as derived from DBC^{2-} → Fe(III) CT band energies and $E_{1/2}$ of Fe(III)/Fe(II) [except for a few complexes like Fe(L13)] and DBSQ/DBC couples, the Lewis acidity decreases in the order Fe(L2) > Fe(L7) > Fe(L1) ≥ Fe(L8) > Fe(L6) > Fe(L5) and thus the observed yield decreases up to Fe(L8) but increases from Fe(L6) and it decreases in the order, Fe(L11) > Fe(L12); Fe(L13) > Fe(L14) > Fe(L15).

For the tripodal ligand complexes, the observed yield for the most Lewis acidic Fe(L10) is four-fold higher than that for Fe(L11); this is consistent with the observation (Whittaker and Lipscomb 1984) that Fe(L10) catalyzes the reaction about 100 times faster than the carboxylate ligand complexes of lower Lewis acidic character. These observations are in line with the finding (Russo *et al* 1986; Que *et al* 1987) that the introduction of a phenolate to the iron coordination environment diminishes the yield of cleavage product. However, the yield for Fe(L12) is five-fold higher than that for



Scheme 2. Substrate activation mechanism proposed for dioxygenase enzymes.

Fe(L11). We suggest that the rate determining step, viz. dissociation of the Fe–O (product) bond is facilitated by the very much decreased Lewis acidity of the iron centre, owing to the two coordinated phenolates. This is supported by the observation that Fe(L15) with two coordination phenolates, like Fe(L12), has almost the same yield. Thus the inclusion of two phenolate groups in the coordination sphere results in a Lewis acidity lower than that, for example, with all-N ligation but appears to be sufficiently Lewis acidic to catalyze the reaction at an adequate rate, as in the enzyme with an active site containing two coordinated phenolates. This is in line with the observation of lower yields for the iron(III) complexes of the above tridentate mono phenolates. The lower yields may be due to the still Lewis acidic five-coordinate DBC^{2-} adducts, which do not encourage dissociation of Fe–O(DBC) bonds and hence the semiquinone character needed for the formation of the peroxide intermediate.

As expected, Fe(L14) shows higher reactivity than Fe(L15). However, the turnover for Fe(L13), in spite of its Lewis acidity being higher than those for Fe(L14) and Fe(L15) and almost the same as that for Fe(L10), is low. This may be ascribed to not only the absence of phenolate coordination to sufficiently decrease the Lewis acidity as illustrated above, but also to the bulky bzim moieties which might offer steric hindrance to substrate binding. This is interesting in view of the prediction from model building (Lipscomb and Orville 1993) based on the structure of the substrate-free PCD enzyme that a single, fairly constrained binding orientation as well as a large rotation of the substrate is essential. However, Fe(L14) shows higher reactivity than Fe(L11) and Fe(L15), both involving comparatively low steric constraints. It appears that the bzim moiety may be involved in stabilising the reactive intermediate by charge delocalisation into the fused benzo ring. The isolation and structure determination of the DBC^{2-} and CAT^{2-} adducts may help to clarify such observations.

Thus, as expected, the Lewis acidity of the iron(III) centre in the complex as well as the dbc and other adducts is modulated by the nature of the donor functionalities of the phenolate ligand. Indeed bzim, py and the introduction of the NO_2 group serve to modulate the Lewis acidity and hence the yield. Thus it is possible to tune and optimize the Lewis acidity of model compounds by incorporating one or two phenolates and other suitable donors in order to increase the turnover as in the enzymes. Such a ligand set would be expected to be close to the one actually selected by nature for the active-site iron. Thus the present results tend to emphasize the role the phenolate ligands play in modulating the Lewis acidity and determining the course of the catalytic reaction.

4. Relevance to iron oxygenases

One of the important conclusions of the present model studies is that the phenolate-to-iron(III) charge transfer band is sensitive not only to the ligand environment but also to the addends and that $CAT^{2-}/DBC^{2-} \rightarrow$ Fe(III) charge transfer band also is remarkable sensitive to the primary ligand environment. When H_2CAT or H_2DBC is added to a solution of the present iron(III) complexes, the absorption band in the region (400–500 nm) of phenolate-to-iron(III) charge transfer was broadened with remarkable increase in absorbance in the range 600–750 nm. This behaviour strikingly resembles that of substrate complexes (Nagy and Lehrer 1972; Nozaki 1974; Bull *et al* 1981; Que 1983, 1985; Pyrz *et al* 1985) of PCD or CTD enzymes in steady state conditions; however, a negligible increase or even a slight decrease of absorption in the 400–500 nm region is observed. Since the present model compounds are coordinatively unsaturated, the binding of DBC or CAT is expected to occur without replacing the already bound donors of the primary ligand. Hence the changes in absorption between 400 and 500 nm may be ascribed to structural changes accompanying DBC binding and/or to the overlap of the high energy $CAT^{2-} \rightarrow$ Fe(III) charge transfer bands with phenolate $\rightarrow$ Fe(III) charge transfer ones. In fact, the difference spectra of the CAT^{2-} and DBC^{2-} adducts confirm these observations. This conclusion is in line with the resonance Raman (Que 1989) and CD spectral studies (Bull *et al* 1981; Que 1983, 1985) carried out on enzyme-substrate complexes. Further, the catechol binding studies have been useful not only in estimating (Viswanathan *et al* 1996) the Δ_0 of complexes but also in diagnosing the coordination environment of the iron site in non-heme iron enzymes which lack iron-phenolate coordination.

The appearance of the $DBC^{2-} \rightarrow Fe(III)$ CT band and the DBSQ/DBC redox wave and the lowering of Fe(III)/Fe(II) redox potential on adding H_2DBC, even in the absence of added base, illustrates the spontaneous deprotonation of the latter on binding to iron. This is consistent with one of the suggested functions of the iron(III) centre in the enzyme, viz. promoting the loss of both the protons of the substrate. This is relevant to the observation that the presence of catechol lowers the potential of the iron(III) centre in the enzyme and renders it difficult to reduce under biological conditions (Pyrz *et al* 1985).

The observation that the yield of the desired cleavage product increases with increase in Lewis acidity of the iron centre in most of the catecholate adducts of the tridentate ligand complexes reflects the coordination of the intermediate peroxide to the metal centre and provides support to the substrate activation mechanism proposed by Que and coworkers. However, for the tripodal ligand complexes the observed yields of desired cleavage product could not be illustrated solely on the basis of the Lewis acidity; it is necessary to assume that the rate-determining step in the substrate activation mechanism proposed by Que and coworkers is the dissociation of the oxygenated product from the iron centre. This is relevant to the suggestion that the slow step of the enzyme reaction is product release and not oxygen attack or ring opening. In fact, more recent studies (Salama *et al* 1978) of the transient kinetics of 3,4-PCD turnover of PCA have revealed the rapid formation of an enzyme-substrate complex.

The EPR spectral features of the DBC^{2-} adducts of the present complexes correspond to high-spin Fe(III) center rather than semiquinone radical. Similarly, the EPR signal observed for the enzyme-substrate complexes may possibly be due to only high-spin Fe(III) rather than to any incipient semiquinone radical. The present iron(III) complexes may be good models also for the iron tyrosinate protein lactoferrin in mimicking its tyrosinate-to-iron(III) charge transfer spectral features and the fairly negative value of $E_{1/2}$.

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