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Solution studies of copper(II) complexes as models for the active site in galactose oxidase

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The complexation of copper(II) (from the perchlorate) by two tripodal ligands H_2L bearing one pyridine and two phenolic arms has been studied in co-ordinating solvents. One of the ligands bears a MeS group at the *para* position of one of the phenolic moieties. In the presence of triethylamine, only brown dimeric neutral complexes $[(CuL)_2]$ have been obtained. In the absence of base, green $[Cu(HL)]^+$ complexes were identified, in which one of the phenolic ligands remains protonated. The brown and the green complexes are reversibly interconverted upon addition of acid or base. Some evidence is given supporting the hypothesis of protonation of the axial tyrosine ligand in the form of galactose oxidase which has been isolated with an exogenous ligand bound to Cu. Electrochemical studies have been performed for the free pro-ligands and for the copper complexes.

Galactose oxidase (GOase) is a secreted mononuclear copper metalloenzyme which catalyses the oxidation of several primary alcohols to aldehydes.[1] The enzyme from the fungus *Fusarium dendroides* has been shown, by X-ray structural analysis at pH 4.5, to have a unique mononuclear copper site in a square-pyramidal co-ordination environment with two histidine imidazoles, two tyrosines (one axial) and one exogenous acetate.[2] The equatorial tyrosine ligand is covalently linked to a cysteine residue by a C–S bond at a position *ortho* to the hydroxyl group (Fig. 1). The active oxidized enzyme harbours a stable tyrosyl free radical on the cysteine-substituted residue which is antiferromagnetically coupled to the copper(II) ion resulting in an EPR-silent complex. The inactive reduced form (≈50% of the native enzyme) shows a copper(II) EPR signal (type II copper protein) and a substrate-reduced form which contains a copper(I) centre.[1] Some copper(II) complexes derived from tripodal tetradentate ligands, containing one or two phenolic and benzimidazole or pyridine ligands, have been described as structural models for the active site of the inactive reduced form of GOase.[3–6] The essentials of the catalytic mechanism of GOase, were elucidated by Whittaker.[1] During submission of our work[7] a model compound derived from a tripodal ligand bearing one (*o*-methylsulfanyl)-*p*-methylphenol group, one *o*,*p*-dimethylphenol group and one 2-(amino-methyl)pyridine group was reported by Whittaker *et al.*[8]

Our own approach for modelling the active site in GOase is in the line with the works of Fenton and co-workers[6] and Whittaker *et al.*[8] We describe here solution studies of the copper(II) complexes derived from two new pro-ligands (depicted in Fig. 1) of the same type as the model of Whittaker *et al.*[8] The first, H_2L^1, bears no substituent at the phenol moieties and the second one, H_2L^2, bears an electron-donating-methylsulfanyl group at one of the two phenol moieties. These two compounds have been designed with the aim of studying the role of the MeS group on the physicochemical properties of the copper complex. This group has been located in the *para* position and not in the *ortho* to avoid the formation of a S–Cu bond.[9] The formation of such a bond was not observed in the last model of Whittaker *et al.*[8] This group in H_2L^2 may be regarded as the cysteine-substituted tyrosine group in the enzyme. The results of our solution studies emphasize the role of the base (added or not) during the complexation of copper by the pro-ligands and evidence the acid–base driven interconversions. This may serve as a model for investigating the deprotonation of the axial tyrosine phenolic group in the enzyme.

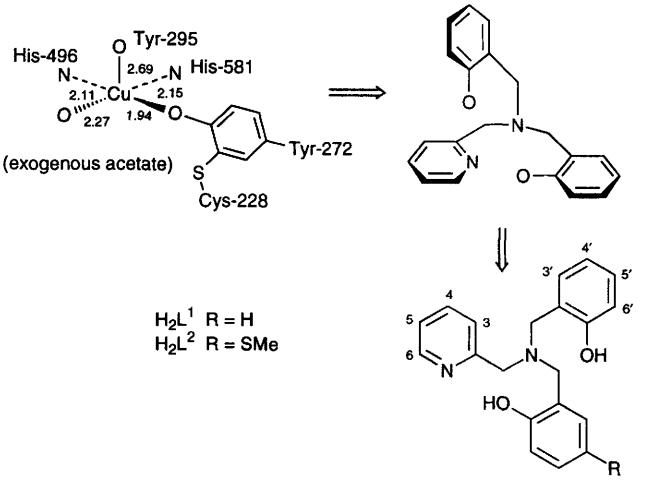

Fig. 1 The active site at pH 4.5 in GOase and possible models for ligands

Experimental

Commercial reagents were used as obtained without further purification. Solvents were purified by standard methods before use. Triethylamine was distilled and stored over potassium hydroxide. Elemental analyses were performed by the CNRS Microanalysis Laboratory of Lyon, France.

CAUTION: although no problems were encountered during the preparation of the perchlorate salts described below, suitable care and precautions should be taken when handling such potentially hazardous compounds.

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Physical measurements

The EPR spectra were recorded at 100 K on a Bruker ESP 300E spectrometer operating at 9.4 GHz (X-band). Quantitative measurements were performed using copper(II) perchlorate solutions as standard (with precautions to avoid power saturation of the EPR signals). Proton and ^{13}C NMR spectra were recorded on a Bruker AC 200 spectrometer, at 25 °C, using $CDCl_3$ as solvent. Chemical shifts were measured in parts per million from internal $SiMe_4$. Fast-atom bombardment (FAB) mass spectra in the positive mode were recorded on a Nermag R 1010C apparatus equipped with a M scan (Wallis) atom gun (8 kV, 20 mA). The peak intensities were normalized to the 3-nitrobenzyl alcohol matrix peak at $m/z = 153.9$, set at 100%. Electronic spectra were obtained using a Uvikon 930 spectrophotometer operating in the range 200–900 nm with quartz cells. The ε values of the absorption bands of the copper complexes are given in $dm^3\ mol^{-1}\ cm^{-1}$ per Cu. Infrared spectra were recorded as KBr pellets (1–1.5%) using a Nicolet IMPACT 400 IR Fourier-transform spectrophotometer (4000–400 cm^{-1}). Magnetic data were collected in the 2–300 K temperature range using a Quantum Design MPMS superconducting susceptometer working at 0.5 T field strength. The SQUID outputs were corrected for the magnetization of the sample holder, and the molar susceptibilities were corrected for the diamagnetism of the constituent atoms using Pascal constants. Electrochemical experiments were carried out using a PAR model 273 potentiostat equipped with a Kipp-Zonen *x-y* recorder. All experiments were run at room temperature under an argon atmosphere. For analytical experiments in millimolar complex solutions a standard three-electrode cell was used. Potentials are referred to a Ag–10 mmol dm^{-3} $AgNO_3$ reference electrode with 0.1 mol dm^{-3} tetrabutylammonium perchlorate as supporting electrolyte in acetonitrile. The potential of the ferrocene–ferricenium couple was 0.07 V under our experimental conditions. Platinum-disc electrodes (5 mm diameter) were polished with 1 μm diamond paste.

Ligand syntheses

Bis(2-hydroxybenzyl)(2-pyridylmethyl)amine (H_2L^1). (2-Hydroxybenzyl)(2-pyridylmethyl)amine (4.28 g, 20 mmol) prepared according to Uma *et al.*[4] and 2-(bromomethyl)phenyl acetate (4.58 g, 20 mmol) prepared according to Karlin *et al.*[10] were refluxed in dry tetrahydrofuran (thf) (100 cm^3) in the presence of triethylamine (2.26 g, 22 mmol) for 24 h under an inert atmosphere. The solution was filtered and the solvent removed *in vacuo*. The crude acetate was hydrolysed with sodium hydroxide (30 cm^3; 40% in water) and ethanol (30 cm^3). Ethanol was removed and HCl was added until neutralization. The compound H_2L^1 was purified by column chromatography on silica gel, yield 90%. It is a white solid, m.p. 173–174 °C. NMR ($CDCl_3$): ^1H (200 MHz), δ 10.78 (s, 2 H), 8.68 (d, J = 4.9, H^6), 7.72 (ddd, J = 7.7, 7.7, 1.7, H^4), 7.30 (dd, J = 7.7, 4.9, H^5), 7.17 (m, 3 H), 7.07 (d, J = 7.4, 2 $H^{3'}$), 6.90 (d, J = 8.2, 2 $H^{6'}$), 6.79 (ddd, J = 7.4, 7.4, 1.0 Hz, 2 $H^{4'}$), 3.90 (s, 2 H) and 3.83 (s, 4 H); ^{13}C, δ 157.3 (s, 2 $C^{1'}$), 155.9 (s, C^2), 148.0 (d, C^6), 137.6 (d, C^4), 130.3 (d, 2 $C^{3'}$), 129.4 (d, 2 $C^{5'}$), 123.4 (d, C^3), 122.61 (d, C^5), 121.51 (s, 2 $C^{2'}$), 119.1 (d, 2 $C^{4'}$), 117.0 (d, 2 $C^{6'}$), 56.35 (t, 2 C) and 55.65 (t, 1 C). FAB^+ mass spectrum: m/z = 322 ($M^+ + 2$), 320 (M^+), 307, 289, 257 (M^+ − methylpyridine), 229 (M^+ − phenol) and 215 (M^+ − methylphenol). IR (KBr): 3037m, 2824m, 2716w, 1585s, 1568w, 1490s, 1381s, 1252s and 749s cm^{-1} (Found: C, 74.6; H, 6.35; N, 8.75. Calc. for $C_{20}H_{20}N_2O_2$: C, 75.0; H, 6.3; N, 8.75%).

(2-Hydroxy-5-methylsulfanylbenzyl)(2-pyridylmethyl)amine. To 2-(aminomethyl)pyridine (1.6 g, 14.8 mmol) in ethanol (20 cm^3) was added 2-hydroxy-5-methylsulfanylbenzaldehyde[11] (2.5 g, 14.8 mmol). To the yellow solution obtained, $NaBH_4$ (1.05 g, 28 mmol in 20 cm^3 ethanol) was added dropwise with cooling on ice. The yellow colour disappeared and the solution was filtered through Celite. The crude product was purified on a silica gel column [elution with ethyl acetate–hexane (50:50)]. Yield 90%. NMR ($CDCl_3$): ^1H (200 MHz), δ 8.59 (d, J = 4.7, H^6), 7.66 (ddd, J = 7.6, 7.6, 1.8, H^4), 7.22 (m, 3 H), 6.99 (d, J = 2.3 Hz, $H^{3'}$), 3.97 (s, 2 H), 3.91 (s, 2 H) and 2.41 (s, SCH_3); ^{13}C, δ 157.4 (s), 156.7 (s), 149.1 (s), 136.5 (s), 129.5 (d), 126.2 (s), 123.0 (s), 122.2 (d), 116.9 (s), 52.7 (s), 51.4 (s) and 18.14 (s).

(2-Hydroxybenzyl)(2-hydroxy-5-methylsulfanylbenzyl)(2-pyridylmethyl)amine (H_2L^2). This compound was synthesized according to the procedure for H_2L^1 using (2-hydroxy-5-methylsulfanylbenzyl)(2-pyridylmethyl)amine (2.7 g, 10.38 mmol), 2-(bromomethyl)phenyl acetate (2.38 g, 1 eqauivalent) and triethylamine (1.6 cm^3). The crude product from the coupling reaction was hydrolysed (see above) and purified by chromatography. Yield 85%. The compound (H_2L^2) is a white solid, m.p. 121–122 °C. NMR ($CDCl_3$): ^1H (200 MHz), δ 10.59 (s, 2 H), 8.57 (d, J = 3.5, H^6), 7.78 (ddd, J = 7.5, 7.5, 1.7, H^4), 7.34 (dd, J = 5.4, 1.7, H^5), 7.24 (d, J = 7.9 Hz, 1 H), 7.12 (m, 4 H), 6.75 (m, 3 H), 3.88 (s, 2 H), 3.78 (s, 2 H), 3.76 (s, 2 H) and 2.38 (s, 3 H); ^{13}C, δ 157.1 (s), 155.9 (s), 155.7 (s), 148.0 (d, C^6), 137.7 (d, C^4), 131.3 (d), 130.4 (d), 130.2 (d), 129.4 (d), 126.6 (s), 123.3 (d, C^3), 122.6 (d, C^5), 122.2 (s), 121.2 (s), 119.1 (d), 117.7 (d), 116.9 (d), 56.4 (t), 56.2 (t), 55.7 (t) and 18.3 (c). FAB^+ mass spectrum: m/z = 367 ($M^+ + 1$), 274 (M^+ − phenol), 259 (M^+ − methylphenol), 228 (M^+ − methylsulfanylphenol), 213, 154, 107 (methylphenol) and 93 (phenol) (Found: C, 68.8; H, 6.0; N, 7.4; S, 8.3. Calc. for $C_{21}H_{22}N_2O_2S$: C, 68.85; H, 6.05; N, 7.65; S, 8.75%).

Copper complexation reactions

$[(CuL^1)_2]$. To a solution of H_2L^1 (1 mmol) in methanol (20 cm^3) were added triethylamine (2 mmol) and $Cu(ClO_4)_2{\cdot}6H_2O$ (1 mmol) and the mixture was stirred at room temperature for 2–3 h; the brown precipitate was filtered off, dried and recrystallized from dichloromethane–methanol by using the diethyl ether vapour-diffusion technique. A brown powder was obtained, yield 96%. FAB^+ mass spectrum: m/z = 764 $\{[(CuL^1)_2 + H]^+\}$, 445 ($[Cu_2L^1]^+$) and 382 ($[CuL^1]^+$) (Found: C, 61.5; H, 4.9; N, 7.1. Calc. for $C_{40}H_{36}Cu_2N_4O_4{\cdot}H_2O$: C, 61.45; H, 4.9; N, 7.15%).

$[(CuL^2)_2]$. This complex was obtained similarly. FAB^+ mass spectrum: m/z = 856 $\{[(CuL^2)_2 + H]^+\}$, 491 ($[Cu_2L^2]^+$) and 428 ($[CuL^2]^+$) (Found: C, 57.2; H, 4.8; N, 6.3. Calc. for $C_{59}H_{68}Cu_2N_4O_4{\cdot}H_2O$: C, 57.7; H, 4.85; N, 6.4%).

$[\{Cu(HL^1)\}_2][ClO_4]_2$. To a solution of H_2L^1 (0.1 mmol) in methanol (10 cm^3) was added 1 equivalent of $Cu(ClO_4)_2{\cdot}6H_2O$. The solution turned green. After stirring for 1 h the solvent was removed and the product recrystallized using the diethyl ether vapour-diffusion technique, yield 67%. The green crystals were unfortunately not suitable for X-ray diffraction measurements. FAB^+ mass spectrum: m/z = 865 ($[\{Cu(HL^1)\}_2ClO_4]^+$), 766 ($[\{Cu(HL^1)\}_2 + H]^+$), 382, 383 $\{[Cu(HL^1)]^+\}$. UV/VIS (CH_2Cl_2): 618 (150), 418 (2330), 270 (8665) and 235 nm (ε = 9180 $dm^3\ mol^{-1}\ cm^{-1}$). IR (KBr): 3628 (phenolic OH), 1658, 1486, 1454, 1274, 1117 cm^{-1} (ClO_4).

$[\{Cu(HL^2)\}_2][ClO_4]_2$. This complex was obtained similarly, (yield 74%). FAB^+ mass spectrum: m/z = 858 $\{[Cu(HL^2)_2 + H]^+\}$, 491 ($[Cu_2L^2]^+$), 428, 429 $\{[Cu(HL^2)]^+\}$ UV/VIS (CH_2Cl_2): 430 nm (ε = 2100 $dm^3\ mol^{-1}\ cm^{-1}$), IR (KBr): 3620 cm^{-1}.

Table 1 Characteristics in methanol (EPR and phenolate to copper c.t. band) for the mononuclear complexes obtained in the presence (brown dimeric) or in the absence of base (green monomeric) $A_{\|}$ in 10^{-4} cm^{-1}, $g_{\|}/A_{\|}$ in cm, λ/nm, ε/dm^3 mol^{-1} cm^{-1}

	Brown complexes		Green complexes				
			EPR				
Ligand*	EPR	UV/VIS λ(ε)	$A_{\|}$	$g_{\|}$	$g_{\perp}$	$g_{\|}/A_{\|}$	UV/VIS λ(ε)
H_2L^1	Silent	414 (2070)	164	2.259	2.061	138	435 (2760)
H_2L^2	Silent	428 (1580)	167	2.280	2.077	138	446 (1850)

* GOase[12] (water): $A_{\|}$ 175, $g_{\|}$ 2.777, $g_{\perp}$ 2.046, $g_{\|}/A_{\|}$ 130, λ 445 (ε 5194).

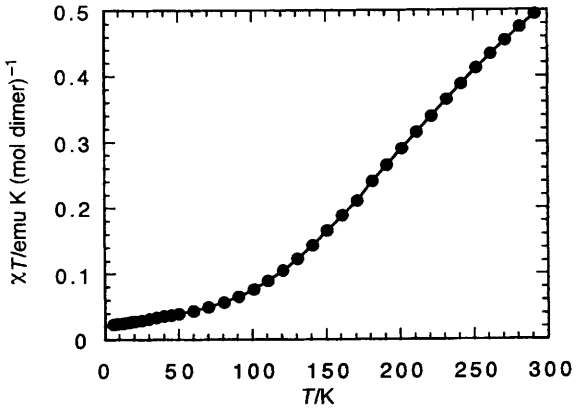


Fig. 2 Plot of χT *vs.* T for solid $[(CuL^1)_2]$. The full line represents the best least-squares fit of the van Vleck equation to the experimental susceptibility data

Results and Discussion

Complexes obtained with the use of triethylamine: EPR-silent dimeric (brown) complexes

When complexation was carried out in the presence of 2 equivalents of triethylamine and using copper(II) perchlorate (*i.e.* a weakly co-ordinating counter anion), the brown dimeric complexes $[(CuL^1)_2]$ and $[(CuL^2)_2]$ were obtained as powders which were characterized by mass spectra and elemental analyses. A variable-temperature magnetic study has been performed for $[(CuL^1)_2]$, revealing a strong antiferromagnetic interaction between the two copper(II) centres (Fig. 2). The susceptibility data were fitted by using the van Vleck expression for molar susceptibility *versus* temperature from the spin-exchange Hamiltonian ($H = -2JS_1 \cdot S_2$) for a pair of spin $S_1 = S_2 = \frac{1}{2}$) and the parameters $g = 2.18$, $J = -189$ cm^{-1} and 4.57% impurity. The temperature-independent paramagnetism = 3.98×10^{-4} cm^3 mol^{-1} was also taken into account.

The complexes $[(CuL^1)_2]$ and $[(CuL^2)_2]$ probably have structures similar to that of the parent compound described by Whittaker *et al.*[8] or Fenton and co-workers[5] with a di-μ-phenoxo unit. Whittaker's complex involves a ligand which is very similar to H_2L^1 and H_2L^2 and exhibits quite similar spectroscopic data in solution [ligand-to-metal charge-transfer (l.m.c.t.) transition and EPR-silent spectrum (see below)]. These complexes retain their dimeric structure in dichloromethane solution (*i.e.* a non-co-ordinating solvent) or in methanol or acetonitrile (more co-ordinating) as shown by the silent EPR spectra, revealing an antiferromagnetic coupling between the two copper ions. The electronic spectra in methanol reveal a broad band at 414 and 428 nm for $[(CuL^1)_2]$ and $[(CuL^2)_2]$ respectively (Table 1). These absorption features are consistent with phenolate-to-copper charge-transfer transitions. The presence of the MeS group on one of the phenolate ligands results in a lower energy for the l.m.c.t. transition.

With tripodal ligands bearing two pyridine and only one phenolic arm, Fenton and co-workers[6] have observed comparable results: the use of copper perchlorate led to dimeric complexes and of chloride (or other co-ordinating anions) to monomeric complexes. Given the presence of only one phenolate moiety (phenolic group deprotonated by triethylamine) leading to a positively charged copper(II) complex it is not surprising if a co-ordinating anion is bound to the copper which is only four-co-ordinated in the cationic complex. Nevertheless the monomeric complexes described by Fenton and co-workers[6] exhibited a marked tendency to dimerize: the mass spectra exhibit peaks corresponding to dinuclear species and the authors emphasized the difficulty in assigning structure with total certainty on the basis of the FAB mass spectrum. With two phenolate moieties, the copper(II) complexes of tetradentate ligands are neutral and do not need counter anions. The co-ordination sphere may be completed to five in three ways: a co-ordinating anion from the copper salt used in the synthesis, a molecule of a co-ordinating solvent, or by dimerization. We have used only the weakly co-ordinating perchlorate counter anion in order to preserve the feasibility of further catalytic reactions (an exchangeable ligand is needed for this purpose). Unfortunately, co-ordinating solvents such as methanol or acetonitrile have been unable to break down the dimeric structures. Whittaker *et al.*[8] have described results very similar to our own (dimeric EPR-silent complex in acetonitrile) and obtained a monomeric complex upon addition of a strongly co-ordinating exogenous pyridine. Uma *et al.*[4] have described a monomeric copper(II) complex obtained with a tripodal ligand of the same type (H_2L^1 with a *p*-nitro group on each phenolic moiety) and *via* a similar synthetic route. This result is surprising (with a ligand of this type bearing a *p*-nitro group at only one of the phenolic moieties, we observed only dimeric species[13]). The copper(II) complexes derived from H_2L^1 and H_2L^2, obtained with the use of triethylamine, are not pertinent models for GOase since they remain dimeric even in co-ordinating media.

Complexation without use of base (green complexes)

The addition of 1 equivalent of copper perchlorate to a methanolic solution of the compounds H_2L^1 and H_2L^2 respectively led to mononuclear complexes as shown by the EPR spectra characteristic of a square-based pyramid (Fig. 3). However, any attempt to isolate the complexes led to the isolation of dinuclear species as powders. The mass spectra revealed peaks corresponding to both aggregated dimeric species and mononuclear species. The IR spectra (KBr pellets) revealed the presence of a phenolic hydroxyl group which is never observed for the complexes obtained in the presence of triethylamine. A variable-temperature magnetic study of the green powder from H_2L^1 revealed only uncoupled copper(II) ($S = \frac{1}{2}$). When the dimeric aggregates (green powder) were redissolved in methanol, spin quantification of the EPR spectra revealed that 100% of the copper was recovered showing that in this medium the green complexes are only mononuclear species (Fig. 3). Table 1 summarizes the main features of the EPR and electronic spectra.

The probable structure of the monomeric complexes in

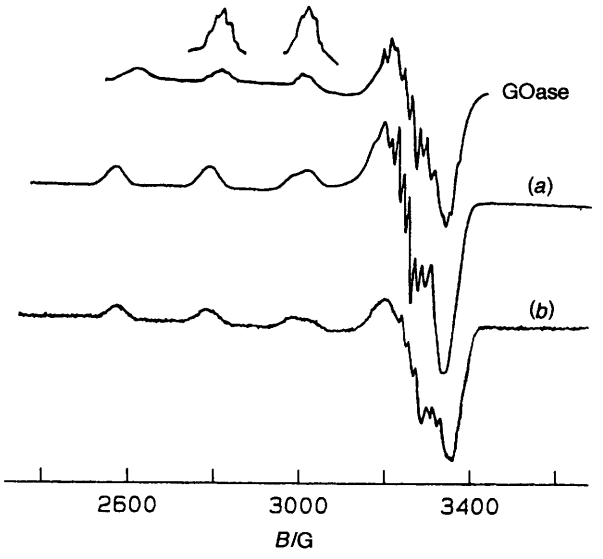


Fig. 3 The EPR spectra at 100 K of GOase,[12] $[Cu(HL^1)]^+$ (*a*) and $[Cu(HL^2)]^+$ (*b*) as perchlorate salts. G = 10^{-4} T

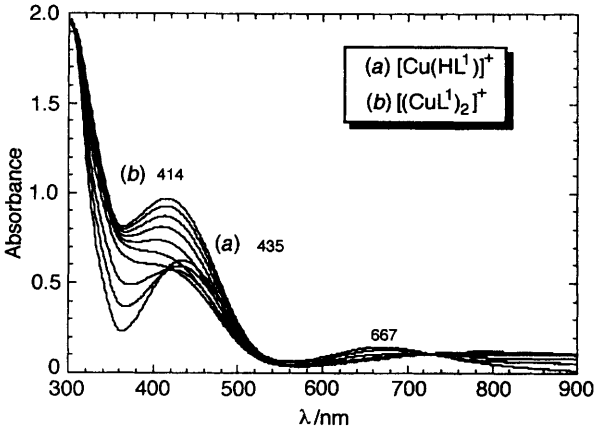


Fig. 4 The change in UV/VIS spectrum upon addition of triethylamine to a 5×10^{-4} mol dm^{-3} methanolic solution of $[Cu(HL^1)]^+$ as its perchlorate salt: (*a*) initial solution, (*b*) after addition of 1 equivalent of triethylamine

solution is depicted in Scheme 1. The presence of both a hydroxyl group (IR spectra) and a phenolate-to-copper c.t. band (electronic spectra) are consistent with this attribution. In the absence of base, only one phenolic group is deprotonated to phenolate upon complexation. The dimeric aggregates may be regarded as monomeric copper(II) complexes associated into an hydrogen-bonded dimeric lattice. This hypothesis is in agreement with Wieghardt and co-workers[12] who have described a mononuclear copper(II) complex containing a co-ordinated phenolate and a phenolic pendant arm: the phenolic group of a one molecule forms a short hydrogen bond to a co-ordinated phenolate oxygen of a second molecule.

The influence of the MeS group on the wavelength of the c.t. band (Table 1) is an argument for the attribution of this band to the substituted phenol subunit of H_2L^2: the unionized phenolic group in the green complex would be the unsubstituted one, while the MeS-substituted phenolic group would give the phenolate ligand responsible for the l.m.c.t. band.

The brown dimeric complexes and the green monomeric complexes are easily interconverted in methanol upon addition of 1 equivalent of perchloric acid to the former or of 1 equivalent of triethylamine (or tetrabutylammonium hydroxide) to the latter. The reversible deprotonation has been monitored by electronic spectroscopy. For instance, Fig. 4 depicts the change from 435 to 414 nm for the c.t. band of the green complex with H_2L^1 upon progressive addition of 1 equivalent of triethylamine. A similar behaviour has been observed for the complex derived from H_2L^2 for which the c.t. band is shifted from 446 to 428 nm. The absence of an isosbestic point rules out the hypothesis of a simple deprotonation step and must represent an additional transformation, most likely the dimerization of the complex.

All the acid–base driven reactions observed in methanolic solution are summarized in Scheme 1 (stereochemical features are arbitrary).

Electrochemical studies

Voltammetric analyses of electroactivity of the free proligands, the protonated monomeric green complexes and the dimeric brown complexes have been performed in acetonitrile at a platinum disc using an Ag–10 mmol dm^{-3} $AgNO_3$ electrode in acetonitrile as reference at a 0.1 V s^{-1} scan rate. The electrochemical data are summarized in Table 2.

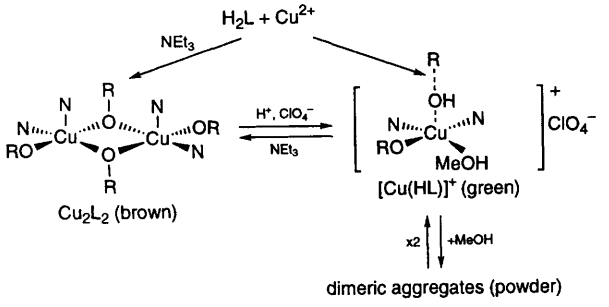
The cyclic voltammogram recorded in a solution of free H_2L^1 exhibits an irreversible oxidation wave at E_{pa} = 0.72 V. This process corresponds to oxidation of the phenolic moieties leading to an unstable radical cation which oligomerizes by radical coupling. The same process is undergone by H_2L^2 at a less positive potential, E_{pa} = 0.42 V, in agreement with the electron-donor character of the MeS group.[14] When the scan limit is extended to higher potentials additional irreversible peaks at E_{pa} = 1.00 and 1.14 V with a shoulder at 0.80 V are observed. This process arises from the oxidation of the non-substituted phenolic groups in addition to that of the MeS group, leading to a sulfoxide derivative. In the presence of tetrabutylammonium hydroxide the phenolic groups are deprotonated and the first oxidation process for the phenolate groups is shifted towards negative potentials as previously reported.[8] The values of E_{pa} = −0.27 and −0.35 V for $(L^1)^{2-}$ and $(L^2)^{2-}$ respectively have been measured.

The oxidation of the brown dimeric complexes takes place at a potential close to that of the protonated free proligands: E_{pa} = 0.77 and 0.44 V for $[(CuL^1)_2]$ and $[(CuL^2)_2]$ respectively (Fig. 5). As previously pointed out by Whittaker *et al.*,[8] metallation parallels protonation in the redox chemistry of this type of ligand. In addition, as for the free proligand, the MeS group in $[(CuL^2)_2]$ shifts the oxidation wave towards less positive potentials. Secondary oxidation of the complex $[(CuL^2)_2]$, occurs at 0.70 V. However, the corresponding wave appears to be the overlapping of several electrochemical processes. In addition, the ligands stabilize the copper(II) state, since reduction of the complexes occurs at very low potentials: E_{pc} = −1.40 and −0.96 V for $[(CuL^1)_2]$ and $[(CuL^2)_2]$ respectively. The reduction process is irreversible for the two complexes and leads to a deposit of copper(0) on the electrode surface, as judged by the observation of a sharp oxidation peak during the reverse scan {E_{pa} = −0.45 and −0.77 V for $[(CuL^1)_2]$ and $[(CuL^2)_2]$ respectively} with the typical features of a redissolution process (Fig. 6).

A very similar electrochemical behaviour has been found for the green monomeric complex $[Cu(HL^1)]^+$ which is characterized by E_{pa} = 0.70 V and E_{pc} = −1.50 V. In contrast, a surprising value for the oxidation potential of $[Cu(HL^2)]^+$ has been found, E_{pa} = 0.81 V, while the reduction process occurs at a similar potential (E_{pc} = −0.90 V) to that observed for $[(CuL^2)_2]$. Taking into account (*i*) the influence of the MeS group on the oxidation potential of the free proligands or of the dimeric complexes and (*ii*) the very similar values for the oxidation potentials of H_2L^1, $[(CuL^1)_2]$ and $[Cu(HL^1)]^+$, an oxidation potential around 0.4 V similar to that of H_2L^2 or $[(CuL^2)_2]$ was expected for $[Cu(HL^2)]^+$. The 0.81 V value remains puzzling.

Table 2 Electrochemical data for the oxidation (E_{pa}) and reduction (E_{pc}) processes of H_2L^1 and H_2L^2 and their copper(II) complexes [determined by cyclic voltammetry in acetonitrile + 0.1 mol dm^{-3} NBu_4ClO_4 at a platinum electrode; scan rate 0.1 V s^{-1}; *E vs.* Ag–10 mmol dm^{-3} $AgNO_3$ + MeCN + 0.1 mol dm^{-3} NBu_4ClO_4

Species	E_{pa}/V	E_{pc}/V
H_2L^1	0.72	—
$(L^1)^{2-}$	−0.27	—
$[(CuL^1)_2]$	0.77	−1.40
$[Cu(HL^1)]^+$	0.70	−1.50
H_2L^2	0.42	—
$(L^2)^{2-}$	−0.35	—
$[(CuL^2)_2]$	0.44	−0.96
$[Cu(HL^2)]^+$	0.81	−0.90



Scheme 1 Acid–base driven reactions in methanolic solution. R = aryl

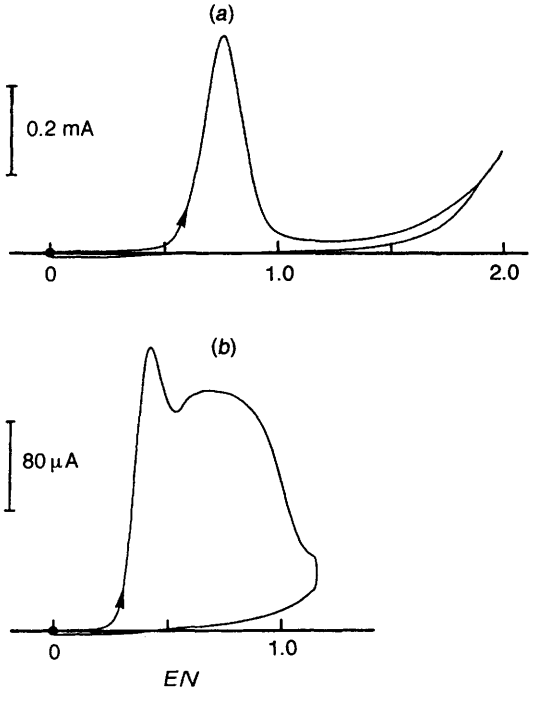


Fig. 5 Cyclic voltammograms (anodic region) of $[(CuL^1)_2]$ (*a*) and $[(CuL^2)_2]$ (*b*), 3.7 mmol dm^{-3}, in MeCN + 0.1 mol dm^{-3} NBu_4ClO_4 at a platinum electrode (5 mm diameter); scan rate 0.1 V s^{-1}; *E vs.* Ag–10 mmol dm^{-3} $AgNO_3$ + MeCN + 0.1 mol dm^{-3} NBu_4ClO_4

Relevance to galactose oxidase

Proton-uptake experiments[1] show that when anions bind to Cu in GOase the phenolate-to-copper l.m.c.t. band is lost and a single proton is taken up by base having $pK_a > 9$. This indicates that the axial tyrosine is deprotonated in the resting enzyme and protonated in complexes where exogenous ligands are bound. The EPR spectra of the green complexes are very similar to that

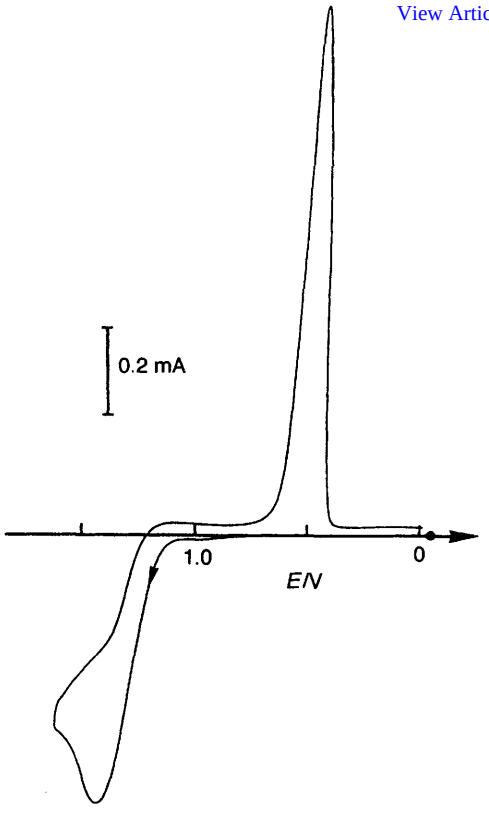


Fig. 6 Cyclic voltammogram (cathodic region) of $[(CuL^1)_2]$, 3.7 mmol dm^{-3}, in MeCN + 0.1 mol dm^{-3} NBu_4ClO_4 at a platinum electrode (5 mm diameter). Details as in Fig. 5

of GOase[15] (Fig. 3). The phenolate-to-copper c.t. bands are in the same range, particularly for the complex of H_2L^2 which is substituted by an MeS group at one of the phenolic subunits, as is the case for GOase. These observations may suggest, for the GOase form for which data are reported, the hypothesis of a protonated axial tyrosyl group with a very weak or no co-ordination of this ligand. This is corroborated by the large copper–oxygen bond length of 2.7 Å for the axial tyrosyl ligand (structure at pH 4.5) or 2.6 Å (pH 7), revealed by the crystal structure of the enzyme.[2] These values are much greater than usual phenolate oxygen–copper bond lengths, *e.g.* 1.94 Å for the equatorial tyrosine group in the enzyme.[2] It is 2.27 Å in a mononuclear copper(II) complex derived from a tripodal ligand of the same type (two pyridyl and one *p*-nitrophenol subunits) in which the phenolate to copper bond is axial.[3] From extended X-ray absorption fine structure measurements, Whittaker and co-workers[16] obtained 1.97 and 1.95 Å for the best-fit average bond lengths of the copper in the inactive and active forms respectively, the copper(II) ion in the active and inactive forms having an apparent co-ordination number of four. The green complexes may be considered as models of the inactive copper(II) form of GOase, when an exogenous ligand is bound.

Conclusion

The compound H_2L^1 and to a greater extent H_2L^1 have been useful to discuss the question of protonation of the axial tyrosine in the active site of the enzyme. They have allowed us to underline the role of the added (or not) base during the preparation of the copper complexes. Easy reversible changes in the co-ordination sphere of the copper ion upon addition of acids or bases have also been evidenced. This emphasizes the importance of the control of pH during solution studies. The propensity for higher aggregation in the monomeric complexes (which has been underlined by Fenton and co-workers[6]) has

also to be taken in account. Electrochemical measurements on the free proligands and on the complexes have evidenced the irreversible formation of an unstable one-electron oxidation product which is an organic radical, undoubtedly of the phenoxyl type. Except in acidic media (and this point remains puzzling), the substitution by MeS stabilizes the phenoxyl oxidation product by several hundred of millivolts. Further investigations are now in progress concerning the reactivity of synthetic models of GOase.

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