Sensitive Valence Tautomer Equilibrium of Paramagnetic Complexes [(L)Cu^{n+}(Q^{n-})] ($n = 1$ or 2; Q = Quinones) Related to Amine Oxidase Enzymes

Jochen Rall, Matthias Wanner, Markus Albrecht, Fridmann M. Hornung, and Wolfgang Kaim*[a]

Abstract: Using the bidentate ligand L = 1-methyl-2-(alkylthiomethyl)-1*H*-benzimidazole with mixed imidazole-N and thioether-S functions, we were able to observe valence tautomer (redox isomer) equilibria [(L)Cu^{II}(Q^{-II})] $\rightleftharpoons$ [(L)Cu^{I}(Q^{-I})] by EPR spectroscopy (Q = alkyl-substituted 3-*tert*-butyl-*o*-benzoquinones). These systems are the first to show such behavior outside amine oxidase enzymes, where the intramolecular electron transfer is essential for the activation of O_2 by copper(I). In contrast, coligands containing exclusively thioether functions yield only copper(I)/*o*-semiquinone radical complexes, while the use of 1,4,7-trimethyl-1,4,7-triazacyclononane (Me_3TACN) gave a structurally and spectroelectrochemically characterized copper(II)/catecholate complex [(Me_3TACN)Cu(Q^3)] (Q^3 = 3,5-di-*tert*-butylcatecholate) in which the square-pyramidally coordinated metal forms one weak and two strong bonds to the three nitrogen donor atoms. Most remarkably, rather small modifications of the quinone shift the valence tautomer equilibrium: the use of slightly more electron-rich methoxy- rather than alkyl-substituted 3-*tert*-butyl-*o*-benzoquinones and L resulted in semiquinone/copper(I) formation exclusively.

Keywords: copper · EPR spectroscopy · isomerizations · quinones · valence tautomerism

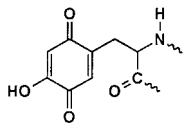
Introduction

Copper-dependent amine oxidases are ubiquituous enzymes which catalyze the oxidation of amines to aldehydes [Eq. (1)].[1–4]

$$R\text{-}CH_2NH_3^+ + O_2 + H_2O \rightarrow R\text{-}CHO + H_2O_2 + NH_4^+ \quad (1)$$

Amine oxidases are typically homodimeric enzymes (M_r = 70 – 90 kDa) with one Cu center per subunit. Their biological roles within the general amine metabolism include developmental functions such as maturation of connective tissue, cross-linking and lignification of cell walls, growth regulation, and cell differentiation; stress response and defence functions through H_2O_2 production are also being discussed.[1–3] Among the biogenic amine substrates for amine oxidases are also neurotransmitters, hormones, and allergens. The two-electron oxidation of primary amines to aldehydes is set off by the two-electron reduction of dioxygen, O_2, to metastable H_2O_2 [Eq. (1)].

The catalysis of a two-electron process such as Equation (1) requires a corresponding catalytic site. Since biological copper usually adopts only the oxidation states I and II, the mononuclear "type 2" copper center[5, 6] in the subunit requires coupling with a redox-active cofactor. For some time, that quinonoid cofactor had been assumed to be pyrroloquinoline quinone (PQQ, methoxatin);[7] however, more recent work has led to a reformulation, revealing the 2,5-quinone form (topaquinone, TPQ) of 2,4,5-trihydroxyphenylalanine as covalently linked, post-translationally modified cofactor.[1–5, 8]



TPQ

The electronic coupling and mechanistic cooperation between the single copper center and the quinonoid cofactor is facilitated by the close proximity of the metal and TPQ, which is evident from structural analysis.[9–11] Protein crystal structures were reported of copper-dependent amine oxidases from procaryotic (*E. coli*) and eucaryotic sources (pea seedling, yeast). They agree in placing the topaquinone and the metal in close proximity in the active site; however, the actual arrangements differ in

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details. In the structure of the enzyme from *E. coli*[9] an "active" crystal form shows triply histidine-coordinated copper $Cu^{II}(His)_3$ with two additional water ligands very close to TPQ, while an "inactive" crystal form contains TPQ directly coordinated to the $Cu^{II}(His)_3$ group through the oxygen atom in the 4-position. In the structure of an enzyme from pea seedlings[10] there is also a loose connection between the metal and the quinonoid ring, suggesting some flexibility in the metal–cofactor interaction. Whereas the triple histidine coordination of the copper center remains a constant feature,[9–11] the obvious flexibility of TPQ with regard to metal bonding is probably crucial for enzymatic catalysis.[10] Recent crystallographic studies[11a] as well as EXAFS results for Cu^{I} and Cu^{II} forms have confirmed the metal–cofactor interaction.[11b]

Incidentally, the very formation of TPQ from tyrosine after translation also requires copper,[3c, 12] as may be anticipated from this metal's role in the hydroxylation of activated aromatics (e.g. by tyrosinase enzymes).[5, 13]

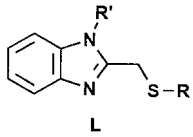
The requirement for a metal such as copper (or a flavin cofactor in metal-free amine oxidases) comes from the necessity to activate the dioxygen cosubstrate in its triplet ground state, $^3O_2$. In biology, there are only two metal states available for this task, namely, high-spin iron(II) and copper(I).[14] The need to generate Cu^{I} starting from the enzyme resting state, which involves Cu^{II} and the aromatic 5-aminated form of the quinone, means that there must be an intramolecular electron transfer [Eq. (2), in which Q: quinone state; $Q^{\bullet-}$: semiquinone state; Q^{2-}: aromatic catecholate state] from the

$$[(L)Cu^{II}(Q^{2-})] \rightleftharpoons [(L)Cu^{I}(Q^{\bullet-})] \text{ (reactive towards } {}^3O_2) \qquad (2)$$

Cu^{II}/"catecholate" form to the Cu^{I}/semiquinone state.[5, 15] Such an equilibrium had indeed been deduced from EPR spectroscopic studies of substrate-reduced forms of amine oxidases from various sources. These studies revealed a low-temperature Cu^{II} EPR signal and a narrow EPR line at higher temperatures; the latter was attributed to a Cu^{I}/semiquinone, that is, an organic radical species.[15] Added cyanide was shown to stabilize the copper(I)/semiquinone form. Detailed studies of enzyme kinetics confirmed that the copper(I)/semiquinone state is a viable intermediate.[3c, 15, 16]

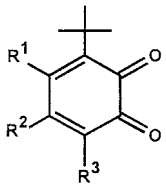
In addition to that crucial intramolecular electron transfer step, the overall enzymatic mechanism[1–5] involves O_2 addition and its reduction by Cu^{I}, the oxidation of the aromatic 5-amino derivative of TPQ to the quinonoid species with formation of H_2O_2 and ammonium ion (deamination step), the reaction of an activated carbonyl group at the generated quinone with the primary amine substrate, and the conversion of the quinoneimine intermediate to the aldehyde and the aromatic form.

Internal electron transfer equilibria ("valence tautomerism", "redox isomerism") involving *ortho*-quinonoid (dioxolene) chelate ligands and transition metals have been discovered and increasingly studied in recent years, especially for cobalt[17] and manganese complexes.[18] However, no such equilibrium between *simultaneously observable* forms has yet been reported for low molecular weight compounds of copper, although the dichotomy between $(Q^{\bullet-})Cu^{I}$ and $(Q^{2-})Cu^{II}$ complexes has long been known.[19–23] Related equilibria[21, 23] were discussed for the dissolution of the structurally characterized solid $[Cu^{I}(NCCH_3)(PPh_3)_2]_2$-$[Cu^{II}(Q^3)_2]$ to $(Q^3)[Cu^{I}(CH_3CN)_n]_2$ and the EPR-detectable semiquinone complex $[(Q_3)Cu^{I}(PPh_3)_2]$ (Q^3 = 3,5-di-*tert*-butyl-*o*-benzoquinone).[21] We therefore addressed the question of a possible Equilibrium (2) by studying through EPR spectroscopy the complexes [(L)-Cu(Q)] with Q = substituted 3-*tert*-butyl-*o*-benzoquinones (Table 1) and the new[24] mixed N/S chelate ligands L = 1-methyl-2-(organothiomethyl)-1*H*-benzimidazoles. In the course of these studies we also investigated the structure and EPR behavior of $[(Me_3TACN)Cu(Q^3)]$ (Me_3TACN = 1,4,7-trimethyl-1,4,7-triazacyclononane and Q^3 = 3,5-di-*tert*-butylcatecholate).



L

Table 1. Quinones used in this study and their redox potentials.[a]



Quinone Q^n	R^1	R^2	R^3	$E_{1/2}$	$r(Cu^{I}/Cu^{II})$[b]
Q^1	H	Me	H	−0.83	< 0.008
Q^2	H	H	*t*Bu	−1.01	0.017
Q^3	H	*t*Bu	H	−1.09	0.086
Q^4	H	OMe	H	−1.11	> 100
Q^5	H	OMe	*t*Bu	−1.14	> 100
Q^6	OMe	OMe	*t*Bu	−1.21	> 100

[a] Values from cyclic voltammetry in THF/0.1 M Bu_4NPF_6 for the $Q/Q^{\bullet-}$ transition. Potentials in V vs. $FeCp_2^{+/0}$. [b] Ratio of copper(I)/semiquinone vs. copper(II)/catecholate form at 300 K in toluene solution.

Experimental Section

Materials: The ligands 1-methyl-2-(methylthiomethyl)-1*H*-benzimidazole (mmb) and the quinones Q^1 = 3,6- and Q^3 = 3,5-di-*tert*-butyl-*o*-benzoquinone, Q^2 = 3-*tert*-butyl-5-methyl-*o*-benzoquinone, Q^4 = 3-*tert*-butyl-5-methoxy-*o*-benzoquinone, Q^5 = 3,6-di-*tert*-butyl-4-methoxy-*o*-benzoquinone, and Q^6 = 3,6-di-*tert*-butyl-4,5-dimethoxy-*o*-benzoquinone were prepared according to literature procedures.[24–26] The derivatives of mmb, 1-methyl-2-(ethylthiomethyl)-1*H*-benzimidazole and 1-methyl-2-(arylthiomethyl)-1*H*-benzimidazoles (aryl = phenyl, 2-tolyl) were synthesized in analogy to mmb from *N*-methyl-1,2-phenylenediamine and the corresponding thioacetic acids.[24]

1-Methyl-2-(ethylthiomethyl)-1*H*-benzimidazole: Yield 75%, colorless crystals, correct C,H,N analysis. ^{1}H NMR ($CDCl_3$): δ = 1.21 (q, 2H, ethyl CH_2), 2.53 (t, 3H, ethyl CH_3), 3.77 (s, 3H, NCH_3), 3.93 (s, 2H, SCH_2C), 7.15–7.32 (m, 4H, CH); ^{13}C NMR (CD_3Cl): δ = 14.3, 25.5, 27.6, 30.1, 109.0, 119.5, 122.0, 122.5, 136.2, 142.1, 151.1.

1-Methyl-2-(phenylthiomethyl)-1*H*-benzimidazole: Yield 46%, colorless needles, correct C,H,N analysis. ^{1}H NMR ($CDCl_3$): δ = 3.73 (s, 3H, NCH_3), 4.36 (s, 2H, SCH_2C), 7.15–7.75 (m, 9H, CH); ^{13}C NMR (CD_3Cl): δ = 30.2, 31.5, 109.1, 119.7, 122.1, 122.7, 127.3, 129.1, 130.8, 134.3, 136.0, 142.3, 150.4.

1-Methyl-2-(2-tolylthiomethyl)-1*H*-benzimidazole: Yield 12%, off-white crystals, correct C,H,N analysis. ^{1}H NMR ($CDCl_3$): δ = 2.32 (s, 3H, tolyl-CH_3), 3.74 (s, 3H, NCH_3), 4.31 (s, 2H, SCH_2C), 7.05–7.75 (m, 8H, CH);

^{13}C NMR (CD_3Cl): $\delta = 20.33$, 30.1, 30.8, 109.1, 119.7, 122.2, 122.7, 126.7, 127.4, 130.3, 131.0, 133.7, 136.0, 142.2, 150.2.

The samples [(L)Cu(Q)] for EPR studies were best obtained by reaction under argon of approximately one equivalent each of Q, L, and activated copper powder in dry toluene or THF. Copper powder was activated under argon atmosphere by treatment with dilute hydrochloric acid, methanol, and finally THF.

[(Me$_3$TACN)Cu(Q)]: A solution of Q (264 mg, 1.20 mmol) in THF (20 mL) was treated with freshly activated copper powder (100 mg, 1.57 mmol). After 1 h the color change to dark blue indicated the formation of the bis-semiquinone complex CuQ_2, whereupon Me_3TACN (200 mg, 1.20 mmol) was added. After 24 h the purplish-blue solution was filtered (to remove excess copper) and reduced to 5 mL. Crystallization at $+4\,^{\circ}C$ gave 515 mg (81%) of blue crystals which could be recrystallized as THF solvate for X-ray diffraction by slow cooling of a saturated THF solution. Anal. calcd. for $C_{23}H_{41}CuN_3O_2 \cdot 0.5\,C_4H_8O$ (527.26): C 61.13, H 9.23, N 8.55; found C 59.41, H 9.24, N 8.67%; UV/Vis (THF): λ_{max} (ε) [nm ($M^{-1}cm^{-1}$)] = 261 (11000), 308 (8700), 496 (80), 625 (20), 1011 (20).

Instrumentation: EPR spectra were recorded in the X band on a Bruker System ESP300 equipped with a Bruker ER035M gaussmeter and a HP5350B microwave counter. A dual mode resonator Bruker ER4116DM was used to study triplet formation. A Bruker AM250 system was employed for the NMR spectra. UV/Vis/NIR absorption spectra were recorded on a Bruins Instruments Omega 10 spectrophotometer. Cyclic voltammetry was carried out in THF/0.1M Bu_4NPF_6 at 100 mV s^{-1} scan rate, with a three-electrode configuration (glassy carbon electrode, Pt counter-electrode, Ag/AgCl reference) and a PAR 273 potentiostat and function generator. The ferrocene/ferrocenium couple served as internal reference. Spectroelectrochemical measurements were performed using an optically transparent thin-layer electrode (OTTLE) cell[27] for UV/vis spectra and a two-electrode capillary for EPR studies.

Crystal structure analysis: Data were collected on a Siemens P4 four-circle diffractometer. The structure was solved by direct methods and refined by full-matrix least-squares on F^2 values for all data (SHELXTL-PLUS and SHELXL-93 programs).[28]

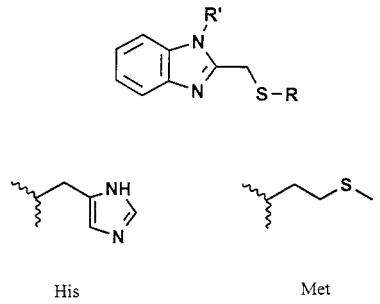
Further details on the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Germany (fax: (+49) 7247-808-666); e-mail: crysdata@fiz-karlsruhe.de), on quoting the depository number CSD-410259.

Results and Discussion

Synthesis of ligands and complexes: It has been shown in several studies[19–23] that the ancillary ligands at copper determine the valence distribution in paramagnetic species [(Q)Cu(L)]. Most nitrogen donor ligands L favor the [(Q^{2-})Cu^{II}(L)] form, whereas typical π-acceptor ligands with soft donor atoms such as triorganophosphanes and -arsanes, carbon monoxide, or isocyanides favor the copper(I)/semiquinone alternative. The EPR spectra of the latter are distinguished by small *g* anisotropy and much reduced metal hyperfine interaction due to the merely indirect contribution from the d^{10} metal center to the spin distribution.

While organophosphanes or -arsanes are not biologically relevant π-acceptor ligands, there is the side chain of the amino acid methionine, the thioether group which can be viewed as a weak π acceptor, for example in heme chemistry.[29] An EPR study involving several thioether species in connection with the 3,5-di-*tert*-butyl-*o*-quinone system has shown that thioether ligation also favors the copper(I)/semiquinone alternative, albeit with slightly increased contributions from the metal to the singly occupied MO.[19, 24a] These contributions are evident from higher than usual *g* factors, slightly decreased semiquinone ligand hyperfine interaction, and fairly large ^{63}Cu and ^{65}Cu isotope hyperfine coupling.

Using the knowledge of this dichotomous[19] behavior we have now employed an ancillary ligand L with a mixed N/S donor set in combination with the well-researched[17, 18, 20, 30] copper/*o*-quinone system. Specifically, we designed a ligand which can form a five-membered metal chelate ring and which offers for coordination one imidazole-imine nitrogen (mimicking histidine) and one saturated thioether function, mimicking methionine (Scheme 1).



Scheme 1.

The ligand of choice, L = 1-methyl-2-(methylthiomethyl)-1*H*-benzimidazole (mmb), had not previously been reported;[24, 31] related compounds are known but were not used as ligands.[32] Compound mmb was obtained[24a] by Phillips condensation between *N*-methyl-1,2-phenylenediamine and (methylthio)acetic acid. A recent structure study has shown that mmb acts as bidentate N,S-chelate ligand to Cu^{I} and Cu^{II} centers to form compounds [(η^2-mmb)Cu(PPh_3)$_2$](BF_4) and [(η^2-mmb)$_2$Cu(η^1-ClO_4)](ClO_4).[24b]

The quinone Q^3 = 3,5-di-*tert*-butyl-*o*-benzoquinone was chosen because of its stability and commercial availability in the quinone state; it has been used most frequently in transition metal/quinone chemistry.[17, 18, 20, 30] After the first detection of a valence tautomer equilibrium with Q^3 [24a] we modified this noninnocent ligand Q^3 to make it slightly more (Q^4–Q^6) or less electron-rich (Q^1, Q^2) (Table 1).

According to the three possible valence-isomeric formulations Cu^{II}/catecholate, Cu^{I}/semiquinone, and Cu^{0}/quinone [Equation (3)], the complexes can be obtained for spec-

$$Cu^0/Q^0 \rightleftharpoons Cu^I/(Q^{\cdot-}) \rightleftharpoons Cu^{II}/(Q^{2-}) \quad (3)$$

troscopic and synthetic purposes in a variety of ways (Scheme 2). In our hands, the reaction of conventionally activated copper powder with the quinone proved to be the best route; it proceeds via a clearly detectable Cu^{II}/(semiquinone)$_2$ complex intermediate.[33]

Structure, EPR and spectroelectrochemistry of [(Me$_3$TACN)-Cu^{II}(Q^3)]: While all attempts to crystallize a copper(I)/semiquinone species failed (dinuclear complexes with semiquinonoid ligands have been structurally characterized[34]), we obtained a crystalline form of the complex [(Me_3TACN)-Cu^{II}(Q^3)], where Me_3TACN is the extensively used and usually symmetrically tridentate 1,4,7-trimethyl-1,4,7-triaza-

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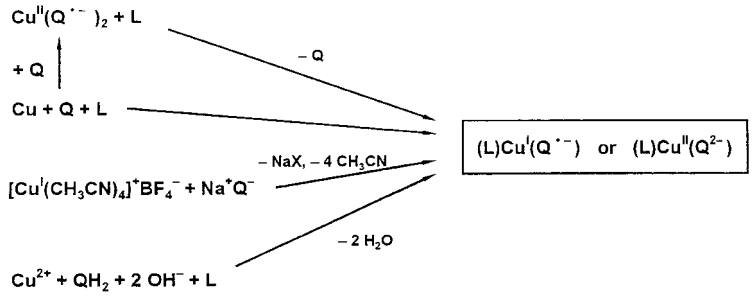


Scheme 2.

cyclononane ligand.[35, 36] Crystals of the THF solvate [(Me$_3$TACN)Cu(Q^3)]·THF were obtained from slow cooling of a saturated THF solution; two crystallographically independent molecules were found in the unit cell. Tables 2 and 3 and Figure 1 summarize the structural data.

Table 2. Crystallographic data for [(Me$_3$TACN)Cu(Q^3)]·THF.

chemical formula	$C_{23}H_{41}CuN_3O_2 \cdot C_4H_8O$	formula weight	527.26
a	882.2(2) pm	space group	$P\bar{1}$
b	1805.3(3) pm	T	183 K
c	1856.3(4) pm	λ	71.073 pm
α	79.60(2)°	ρ_{calcd}	1.242 g cm^{-3}
β	87.122(13)°	μ	0.805 mm^{-1}
γ	75.891(10)°	$R1[I > 2\sigma(I)]$	0.0514
V	2.8202(9) nm^{-3}	$wR2$(all reflns)	0.1700
Z	4	GoF	1.055

Table 3. Selected bond angles(°) for [(Me$_3$TACN)Cu(Q^3)]·THF.

O12-Cu1-O11	87.30(10)	O22-Cu2-O21	87.11(10)
O11-Cu1-N11	94.77(11)	O21-Cu2-N21	94.33(11)
O12-Cu1-N12	92.77(11)	O22-Cu2-N22	93.73(11)
N11-Cu1-N12	84.61(12)	N21-Cu2-N22	84.40(12)
O12-Cu1-N13	100.22(12)	O22-Cu2-N23	97.78(12)
O11-Cu1-N13	108.76(13)	O21-Cu2-N23	113.27(11)
N11-Cu1-N13	81.83(12)	N21-Cu2-N23	83.30(12)
N12-Cu1-N13	82.99(13)	N22-Cu2-N23	81.89(12)

The molecules of [(Me$_3$TACN)Cu^{II}(Q^3)] show no special intermolecular interaction in the crystal. The distances in the O-C-C-O section of the coordinated dioxolene ligand are typical for the catecholate state,[20, 35] as supported by the previously reported[19] copper(II) EPR features of the complex in solution. The metal itself exhibits a tetragonal-pyramidal coordination (Figure 1), so one of the three originally equivalent N-donor positions is placed in the axial position at a rather long distance, about 0.2 Å longer than for the nitrogen centers in the basal plane. (TACN ligands cover triangular faces,[36] here of a tetragonal pyramid.) Similar symmetry-breaking of a TACN ligand has been observed in dinuclear OH-bridged complexes of copper(II)[37] and in very recently reported structures[35] containing copper(II), 1,4,7-tribenzyl-1,4,7-triazacyclononane, and tetrachloro-*o*-benzoquinone in either the singly or doubly reduced state.

An important aspect of compound [(Me$_3$TACN)Cu^{II}(Q^3)] was discovered when an EPR spectrum was recorded at 110 K in glassy frozen dilute THF solution, especially using the dual-mode resonator. These experiments reveal half-field EPR signals (Figure 2) which indicate the population of a triplet state and thus suggest at least partial dimerization of [(Me$_3$TACN)Cu^{II}(Q^3)] in solution. A similar phenomenon was reported for [(bpy)Cu^{II}(Q^3)], where a structure of the dimer with pentacoordinate (bridging) copper atoms was obtained.[30a] Since monomeric, isolated [(Me$_3$TACN)Cu^{II}(Q^3)] already has a pentacoordinate copper center in the solid state, we can envisage two alternatives: one would imply a dimer with two six-coordinate metal centers in a rather crowded

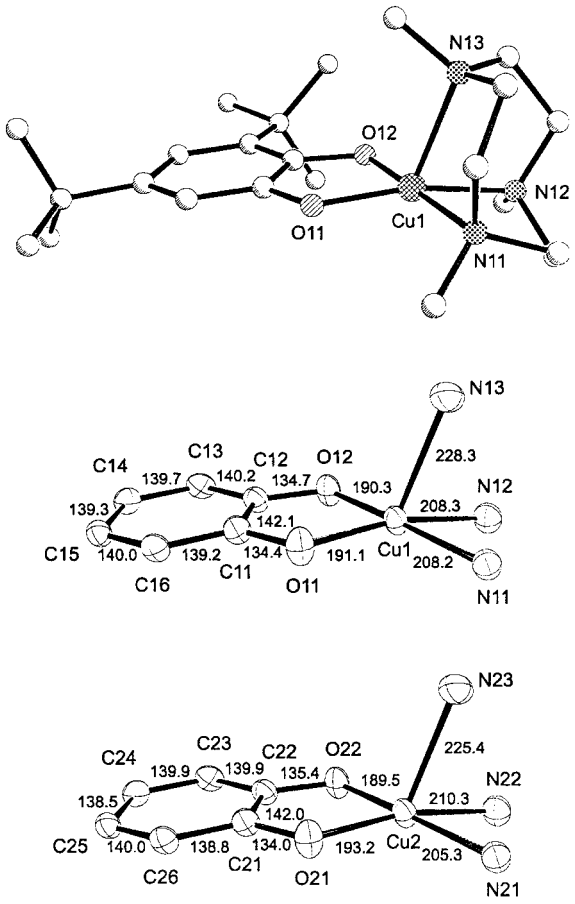


Figure 1. Structure and essential distances in two crystallographically independent molecules of [(Me$_3$TACN)Cu(Q^3)]·THF (e.s.d. < 0.8 pm). For angles, see Table 3.

arrangement, the other implies dissociation of the already "loosened" N13 and N23 of Me$_3$TACN and dimerization through two pentacoordinate copper(II) centers as in [(bpy)-Cu(Q^3)]$_2$.[30a] We shall pursue this question further, also hoping to obtain the structural information necessary for a more detailed analysis of the triplet EPR signals.

Complex [(Me$_3$TACN)Cu^{II}(Q^3)] is reversibly oxidized at −0.68 V vs. Fc$^{+/0}$ in THF/0.1M Bu$_4$NPF$_6$ to an EPR-silent[30d] copper(II)–monosemiquinone complex. This oxidation could

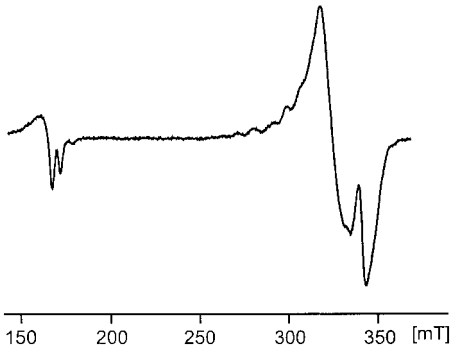
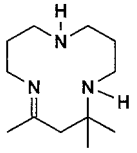


Figure 2. EPR spectrum of $[(Me_3TACN)Cu(Q^3)]$ in glassy frozen THF solution (110 K), using a dual mode resonator ($B_1 \perp B_2$).

be monitored spectroelectrochemically in an OTTLE cell. It is accompanied by several emerging bands in the visible-to-near-infrared region: λ_{max} (ε) [nm ($M^{-1}cm^{-1}$)] = 305 (12000), 379 (780), 448 (550), 573 (350), 797 (170), 900 sh. These values and the data for the parent catecholate complex (see Experimental Section) are rather similar to those reported for corresponding $Cu(Q)^{+/0}$ complexes with the potentially tridentate 2,4,4-trimethyl-1,5,9-triazacyclododec-1-ene macrocyclic ligand **1**,[30d] confirming the postulated pentacoordination.



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Distinctly different absorption spectra were reported for copper(II)–semiquinone complexes with bidentate N-donor ligands and non-pentacoordinate copper(II);[30c] these spectra resembled more closely the spectra of the free semiquinone $(Q^3)^{\bullet -}$ in THF, which shows a broad band at 654 nm (ε 460 $M^{-1}cm^{-1}$) and additional features at 384 (2250), 346 (2160), and 317 nm (5660 $M^{-1}cm^{-1}$). The catecholate $(Q^3)^{2-}$, on the other hand, does not exhibit prominent absorption bands above 350 nm. Further oxidation of $[(Me_3TACN)Cu(Q^3)]^+$ to a copper(II)–quinone complex occurs irreversibly at $E_{pa} = +0.36$ V vs. $Fc^{+/0}$, reduction of $[(Me_3TACN)Cu(Q^3)]$ is also irreversible at $E_{pc} = -2.32$ V vs. $Fc^{+/0}$ (100 mV s^{-1} scan rate).

EPR spectroscopic study of the valence tautomer equilibrium $[(L)Cu^{n+}(Q^{n-})]$, $n = 1,2$: Reaction of the mixed N/S donor ligand L = mmb with copper powder and 3,5-di-*tert*-butyl-*o*-benzoquinone Q^3 in either THF or toluene yielded a brown solution which displayed an EPR-detectable (Figure 3) temperature-dependent valence tautomer equilibrium, as observed previously only for amine oxidase enzymes.[15]

The best spectroscopic results were obtained in toluene solution, which offers a very wide range of the liquid state, forms glassy frozen solutions for "powder" EPR spectra, and induces little dissociation of the complex due to the absence of heteroatom donor centers.

Just as in the enzyme,[15] the low-temperature form is the Cu^{II}/catecholate state. In THF or toluene, that form of $[(L)Cu^{II}(Q^3)]$ exhibits typical[38] Cu^{II} EPR features (Figures 3, 4): $g_1 = 2.248 \pm 0.003$, $g_{2,3} = 2.058 \pm 0.002$, $A_1 = 18.6 \pm 0.1$ mT (^{63}Cu), $A_1 = 20.0 \pm 0.1$ mT (^{65}Cu), $A_{2,3}(^{63,65}Cu) = 2.7 \pm 0.1$ mT;

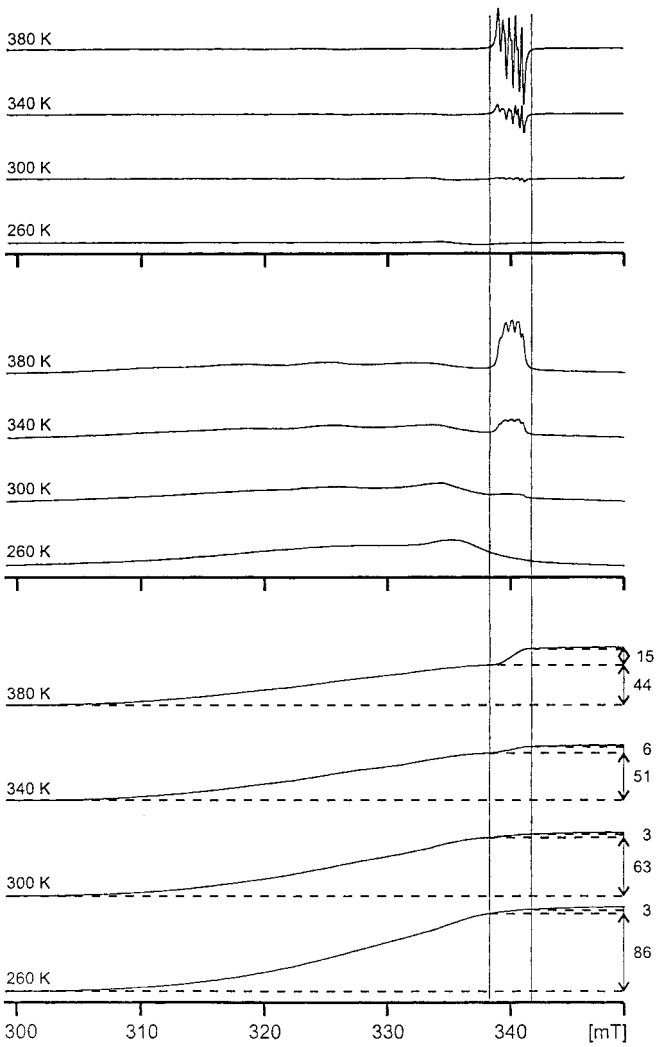

Figure 3. EPR spectra of the solution obtained from copper, 3,5-di-*tert*-butyl-*o*-quinone, and 1-methyl-2-(methylthiomethyl)-1*H*-benzimidazole in toluene at different temperatures: first-derivative spectra (top), singly integrated derivative spectra (center), doubly integrated spectra (bottom).

$g_{iso} = 2.120 \pm 0.003$, $A_{iso} = 8.5 \pm 0.1$ mT and an additional isotropic ^{14}N hyperfine coupling of 1.0 ± 0.1 mT from *one* coordinated nitrogen donor atom as best observed in THF. This latter observation rules out the coordination of two ligands L through their respective nitrogen donor centers. At temperatures above 250 K, this copper(II) EPR signal is beginning to be replaced by an isotropic Cu^I/semiquinone EPR signal at $g = 2.0052$ which completely dominates above 350 K in toluene (Figures 3, 5). It can be simulated with the typical[19, 24a] set of coupling constants $a(^{63}Cu) = 0.51$ mT, $a(^{65}Cu) = 0.54$ mT, $a(^1H) = 0.30$ mT (H^4 proton of one-electron reduced Q^3). A similar result was obtained when the modified ligand L′ = 1-methyl-2-(ethylthiomethyl)-1*H*-benzimidazole was employed in $[(L')Cu^I(Q^3)]$ (Table 4). 1-Methyl-2-(arylthiomethyl)-1*H*-benzimidazoles did not react to EPR-active species.

Double integration of the first-derivative EPR signal was performed (Figure 3) to estimate the ratio between the paramagnetic species. Considering the very different appearances and linewidths of the two subspectra we may yet

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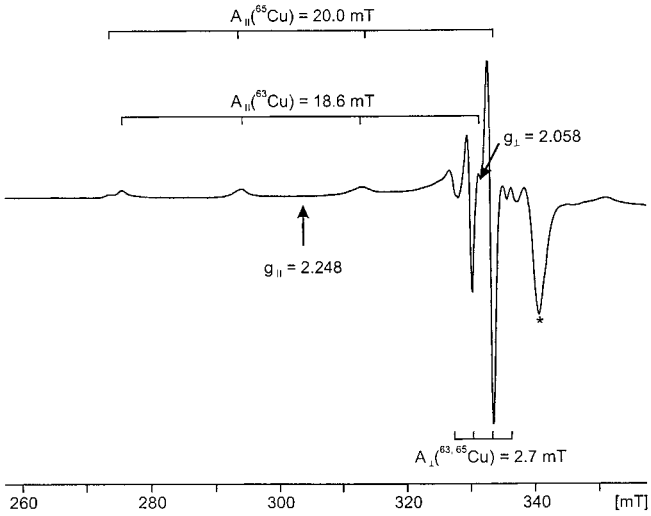


Figure 4. EPR spectrum of [(mmb)Cu(Q^3)] in glassy frozen toluene solution (110 K); *: forbidden transition (see ref. [38]).

Table 4. *g* Factors and EPR hyperfine data[a] for copper(I) semiquinone complexes in toluene.

Semiquinone complex[b]	*g*	*a*(^{63}Cu)	*a*(^{65}Cu)	*a*(^{1}H)[c]	*T*
[(L)Cu(Q^1)]	2.0048	0.51	0.54	0.34 (1 H)	370
[(L)Cu(Q^2)]	2.0055	0.53	0.56	0.33 (2 H)	320
[(L)Cu(Q^3)]	2.0052	0.51	0.54	0.30 (1 H)	380
[(L′)Cu(Q^3)]	2.0054	0.56	0.60	0.32 (1 H)	380
[(L)Cu(Q^4)]	2.0046	0.41	0.44	0.34 (1 H)	340
[(L)Cu(Q^5)]	2.0047	0.41	0.44	0.36 (1 H)	330
[(L)Cu(Q^6)]	2.0047	0.39	0.42		330

[a] From simulated spectra; $g \pm 0.0001$, coupling constants $a \pm 0.02$ mT. [b] L = mmb; L′ = 1-methyl-2-(ethylthiomethyl)-1*H*-benzimidazole. [c] Hyperfine coupling of *tert*-butyl, methyl, or methoxy protons; substituents in the 6 position were too small to be determined.

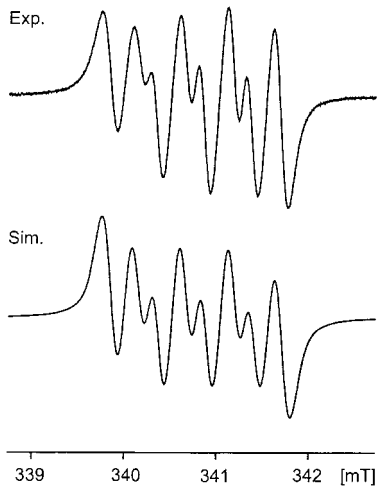


Figure 5. EPR spectrum of [(mmb)Cu(Q^3)] in toluene (380 K) with computer simulation (linewidth 0.19 mT).

conclude that the copper(II)/catecholate signal dominates even at higher temperatures where the copper(I)/semiquinone signal with its sharp lines appears as the most prominent species. However, it is not unexpected that—as in the enzyme[15, 16]—the Cu^{II}/catecholate state represents the low-energy form. As the derivative spectrum (Figure 3) and its integrated forms illustrate, the valence tautomer equilibrium [Eq. (2)] is a completely reversible process unless prolonged heating at temperatures above 360 K causes notable decomposition for the prototypical system [(L)Cu(Q^3)].

Employing the other five 3-*tert*-butyl-*o*-benzoquinones from Table 1 with their slightly different substitution pattern, we observed qualitatively similar but not quantitatively identical (Tables 1, 5) valence-tautomer behavior for the alkyl-substituted systems [(L)Cu(Q^1)] and [(L)Cu(Q^2)]. Table 5 contains information for the systems in two different solvents. Given the obvious limitations of the approach and of the integration procedure (experimental error margins of about 10%), the linear correlation between EPR intensity ratios and T^{-1} gives values corresponding to energies between 5 and 25 kJ mol^{-1} for the enthalpy change associated with reaction (2).

Table 5. Valence tautomer characteristics of copper–quinone complexes.

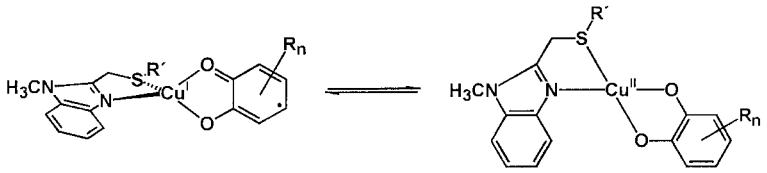
		Linear regression parameters[a]		
System	solvent	*A*	*B*	R^2 (*n*)
[(L)Cu(Q^1)]	toluene	−729.1	−0.540	0.950 (4)
[(L)Cu(Q^1)]	THF	−1120.2	+1.224	0.986 (5)
[(L)Cu(Q^2)]	toluene	−1081.6	−0.448	0.991 (5)
[(L)Cu(Q^2)]	THF	−2638.8	+4.063	0.975 (4)
[(L)Cu(Q^3)]	toluene	−1276.4	+2.181	0.894 (4)
[(L)Cu(Q^3)]	THF	−2902.9	+6.154	0.995 (3)

[a] For equation $\ln\{I_{Cu_I}/I_{Cu_{II}}\} = A \cdot T^{-1} + B$; *I*: integrated amount of copper(I) or copper(II) forms.

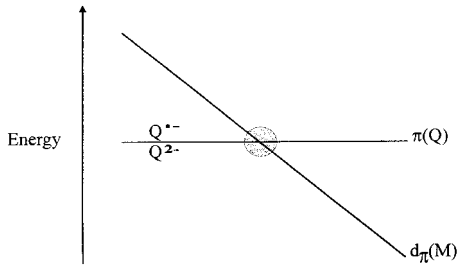
The three methoxy-substituted species, however, yielded only the semiquinone form [(L)Cu^I(Q)], Q = Q^4–Q^6, as EPR-detectable valence combination with the typical[19] copper(I) semiquinone hyperfine values (Table 4). Disregarding steric effects, there is an approximate correlation between the redox potential of the quinone and the amount of the copper(I)–semiquinone alternative present in the equilibrium (Table 1): the more negative the potential and thus the electron donor capacity of Q, the higher the amount of copper(I)–semiquinone formed in the valence tautomer equilibrium. Table 5 also illustrates a revealing solvent effect; the more polar THF favors the more "ionized" copper(II)–catecholate form compared to the copper(I)–semiquinone alternative.

Although the detailed coordination environment of copper in amine oxidases[8–10] is different from the one invoked here, the functional mimicking of the valence tautomer equilibrium employing biochemically relevant ligand donor centers points to one possible mechanism for switching between nonactive (Cu^{II}) and dioxygen-activating states (Cu^I). One possibility would be a polarity change of the environment of the active site, as suggested by the results shown in Table 5. A different and more enzyme-inherent mechanism would be a conformational change, shifting the geometry of the copper center from a square-pyramidal structure (optimum for Cu^{II}) to a more tetrahedral arrangement that would make the Cu^I state better accessible. The large Cu^I/Cu^{II} structural difference between the corresponding minimum-energy configurations is prob-

ably responsible for the simultaneous detection of two slowly interconverting different species in equilibrium (Scheme 3), without significant orbital mixing (covalency).[20, 39] Nevertheless, the valence tautomer equilibrium is quite sensitive to the environment (Tables 1, 5), the region of orbital crossing (Scheme 4)[20] being rather small.



Scheme 3.



Scheme 4.

To summarize, then, this report describes the first successful spectroscopic simulation of the intriguing EPR spectroscopic response of an important class of enzymes by means of a systematically designed low molecular weight coordination arrangement. There is good spectroscopic correspondence between the system presented and the behavior of amine oxidases, including the presence of the copper(II)/catecholate form as the low-energy state. However, there are also obvious differences. One concerns the quinone, which in our case functions as (chelating) *o*-quinone and thus confers stability in the absence of a protein scaffold; the other relates to the ligand L, which contains a thioether function despite the lack of sulfur groups close to the active site of the enzyme. Our future efforts will thus be directed at attempts to obtain a crystalline form of a valence tautomeric system, even though previous results[21, 22f] suggest that crystallization tends to trap the copper(I) or copper(II) states.

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