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Cooperation of metals with electroactive ligands of biochemical relevance: Beyond metalloporphyrins*

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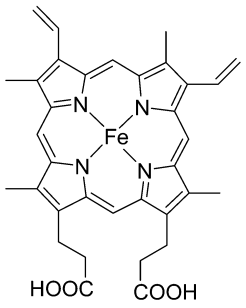
Abstract: In addition to the widely studied biometal complexes of tetrapyrrole ligands such as hemes (Fe), cobalamins (Co), and factor F430 (Ni), there are other, more recently established systems in which transition metals and redox-active cofactors such as pterins, flavins, quinones, or phenoxyl radicals cooperate in electron transfer and substrate activation. The cases of the molybdenum or tungsten containing oxotransferases involving pyranopterin as essential ligand and the copper-dependent quinoproteins such as amine oxidases will be discussed. The structural and functional description of these systems will be complemented by results from model studies.

INTRODUCTION

Organic and inorganic components have coevolved in organisms to perform physiological functions in an optimized way. A well-recognized case is the versatile heme system [1] (Scheme 1) in which the porphinoid macrocyclic ligand not only modifies the metal in terms of spin state and reactivity, as a typical "non-innocent" ligand [2] it can also participate directly in electron transfer, with or without concomitant reactions of the central metal. This added functional potential can create ambiguity as to the appropriate oxidation state description because of possible intramolecular electron transfer; a formal series of "valence tautomeric" (or "redox isomeric") [3] species is illustrated in Scheme 2.



heme
(P^{2-})-Fe^{II} form

Scheme 1

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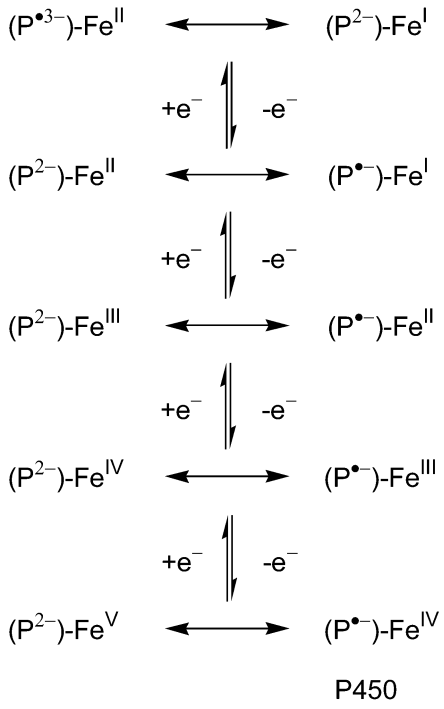
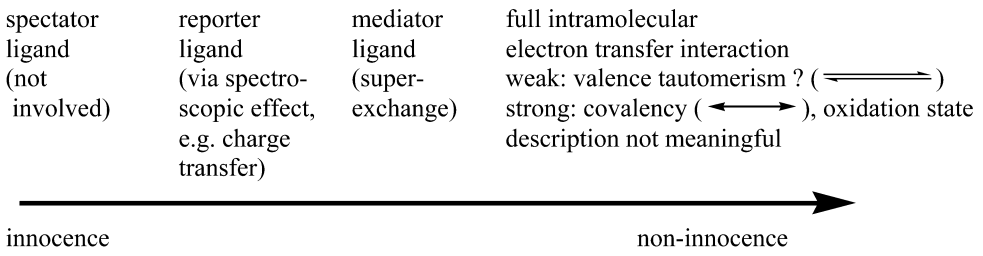


Scheme 2

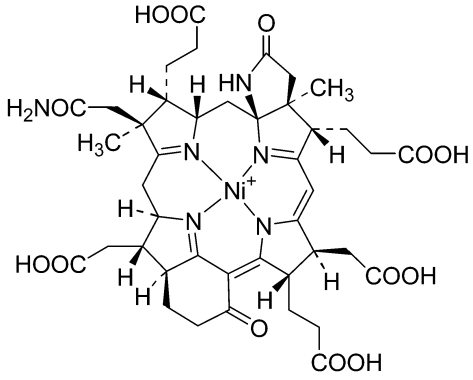
The general extent of involvement of a ligand in charge- or electron-transfer interaction with a metal center is outlined in Scheme 3, which illustrates the array of options for any particular case.



Scheme 3

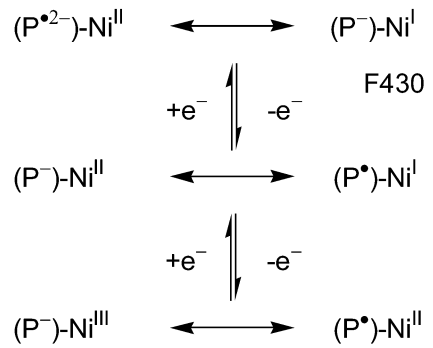
Scheme 2 does not include the additional alternatives resulting from spin isomerism [1] or axial non-innocent (substrate) ligands such as $NO^{+/\bullet/-}$, $O_2^{o/\bullet-/2-}$, or $O^{2-/\bullet-}$, which can further add to the variety of oxidation-state alternatives. Important examples for the latter include nitric oxide synthase (NOS) [4], myoglobin (*Mb*), and hemoglobin (*Hb*) [5], and the highly oxidized intermediate forms of heme peroxidases and P450 systems which may involve the one-electron oxidized porphinato ligand [6,7]. Although the ligand then is in effect an anion radical (the porphinato ligand is normally dianionic in metalloporphyrins), the arrangement $[(P^{\bullet-})Fe^{IV}O]^+$ is often referred to as "radical cation" [7]. However, the unsaturated porphyrin macrocycle may "buffer" electron density both ways, the one-electron reduced ($P^{\bullet 3-}$) state being well known in the form of reduced chlorophylls following the primary processes of photosynthesis [8].

A similar electron transfer ambiguity has been discussed in connection with nickel complexes of porphinoid ligands related to the function of factor F430 (Scheme 4) in methane-releasing coenzyme M reductase [9]. Compounds of the corresponding series in Scheme 5 have been probed by electron paramagnetic resonance (EPR) spectroscopy and calculations for model systems [10].



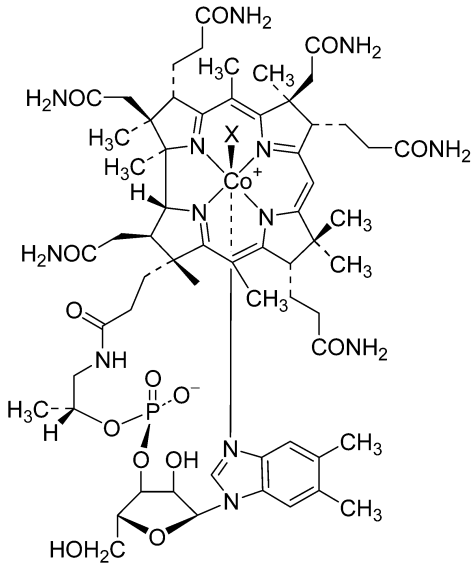
coenzyme F430

Scheme 4



Scheme 5

For the cobalt–corrin interaction in cobalamins (Scheme 6) it is definitely the metal which undergoes the stepwise electron transfer between Co^{I} and Co^{III} [11], however, corrinoid (corrole) ligands in metal complexes can clearly undergo electron-transfer reactions of their own [12].

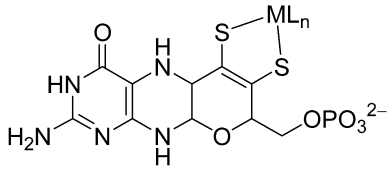


Scheme 6

Whereas the tetrapyrrole ligands are among the most obvious redox-active nonsubstrate ligands in biochemistry, there have been other such biological ligands discovered during the last 15 years for oxidase enzymes. One involves a very special pyranopterin heterocyclic system (Scheme 7) with a potentially redox-active α-dithiolene chelate coordination site (Scheme 8) for molybdenum or tungsten in oxotransferase enzymes (1) [13,14].

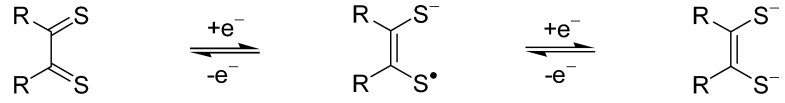
$$E + H_2O \rightleftharpoons EO + 2\,H^+ + 2e^- \quad (1)$$

E = NO_2^-, SO_3^{2-}, R_2S, AsO_2^-, HCO_2^-, RCHO, xanthine



M = Mo or W

Scheme 7



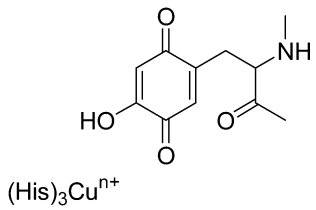
1,2-dithione
(α-dithiolene)

ene-1,2-dithiolate(2-)

Scheme 8

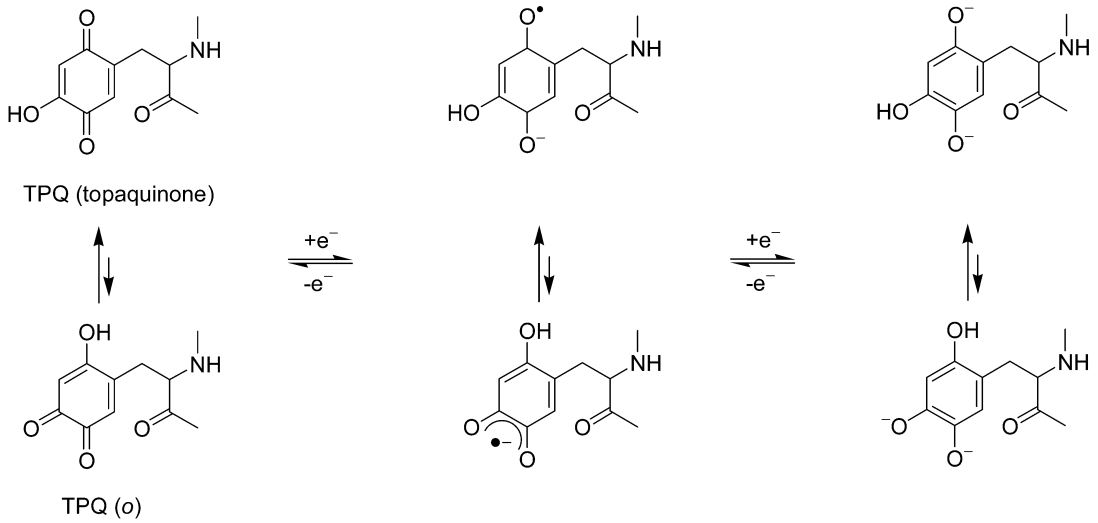
The other example concerns copper-dependent quinoproteins such as amine oxidases (Scheme 9) which include *ortho*/*para*-quinone redox systems (Schemes 10,14) to effect a two-electron transformation (eq. 2).

$$RCH_2NH_2 + O_2 + H_2O \longrightarrow RCHO + H_2O_2 + NH_3 \quad (2)$$



copper/topaquinone arrangement
in amine oxidases

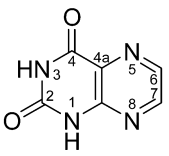
Scheme 9



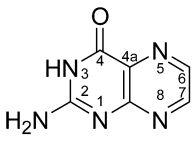
Scheme 10

OXOTRANSFERASES

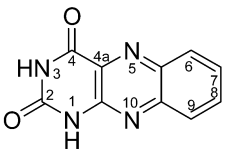
The molybdenum- and tungsten-containing oxotransferases include a number of essential enzymes such as sulfite, xanthine, and aldehyde oxidases, nitrate reductase or formate dehydrogenases (eq. 1) [13,14]. These enzymes are remarkable because they rely on the only known examples of essential 4d (Mo) or even 5d (W) elements in biochemistry [18]. While the two elements are quite bioavailable, their requirement indicates a strong need for the particular two-electron reactivity associated with these two elements under physiological conditions. Equally unusual is, however, the use of one or two pyranopterin ligands (Scheme 7) [13], the exact structure of which has been elucidated only through protein X-ray crystallography of a W-containing aldehyde oxidase from *Pyrococcus furiosus* [19]. This remarkable ligand contains *two* redox-active sites, the metal-binding enedithiolate part (Scheme 8) and the pterin section. Like the related alloxazines, isoalloxazines (flavins) and lumazines (Scheme 11) [20,21], the pterins have an elaborate proton/electron transfer chemistry of their own as illustrated in Scheme 12 [21,22].



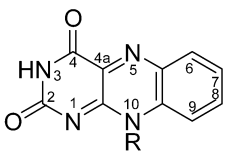
lumazine



pterin

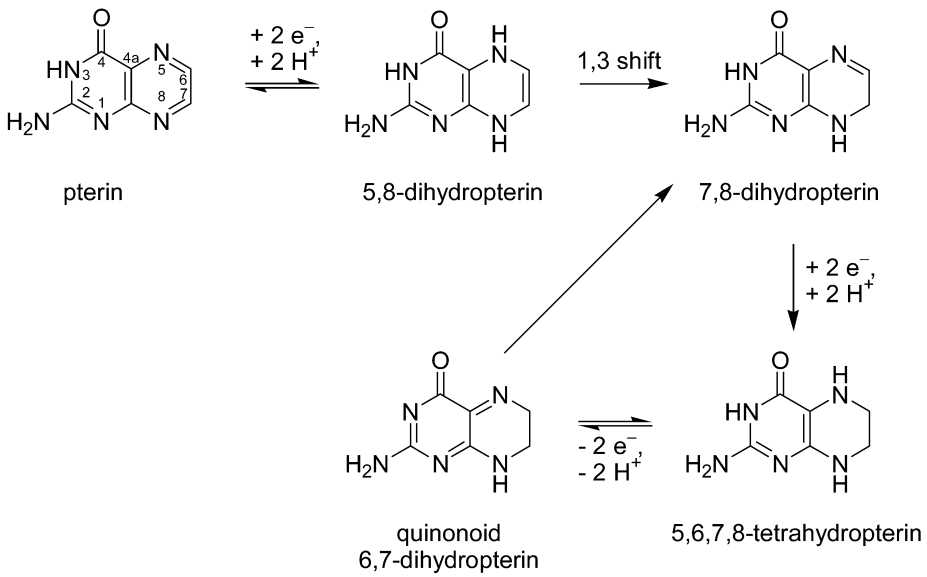


alloxazine



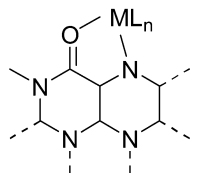
isoalloxazine (→ flavíns)

Scheme 11



Scheme 12

Tetrahydro forms like tetrahydrofolic acid or tetrahydrobiopterin are of immediate biochemical relevance as cofactors in biosynthetic processes [22,23], the various dihydro isomers and some radical species are important intermediates [22]. All heterocycles of Scheme 11 and their radical forms can potentially bind metals, especially at the O4,N5 chelate site (Scheme 13) [24], making them good candidates for model compounds [25].



Scheme 13

However, the Mo and W complex fragments ML_n of actual oxotransferase enzymes (L = O, S, OR, SeR) [13,14] are coordinating the pyranopterin through the enedithiolate chelate configuration at the specially formed tetrahydropyrane ring. The coordination chemistry of α-dithiolenes/enedithiolates has been extensively studied in the 1960s [26] because these molecules are strongly interacting non-innocent ligands [2], leaving oxidation-state assignments often uncertain because of metal/ligand orbital mixing (covalency) [27,28].

Reduced pterins and the related dihydroflavins containing a reduced isoalloxazine ring are generally known to interact with metal sites (Fe, Cu) in proteins to effect oxidoreductase activity. Examples include nitric oxide synthase [22] and aromatic amino acid hydroxylases [29] such as phenylalanine hydroxylase, tryptophane hydroxylase, and tyrosine hydroxylase [30]. While a direct coordinative contact is usually not established, the chelating protein scaffold and hydrogen bond interactions may provide pathways for electron transfer. It should be noted that dihydroflavins and tetrahydropterins are well-established centers of O_2 activation at the ring-connecting 4*a* carbon atom [22,30,31].

The function of the partially saturated and thus nonplanar [13] pyranopterin heterocyclic system with its elaborate biosynthesis [32] lies in connecting the presumed catalytic center ML_n (Mo or W) with the extended intraenzymatic electron-transfer chain involving two separate iron-sulfur clusters and the FAD/$FADH_2$ system [13,14]. The metal-enedithiolate interaction usually involves strong electronic coupling approaching covalency, causing large effects on the thermodynamics (redox potential) [28], direction and kinetics of electron transfer [27]. While the metalladithiolene function is not π conjugatively connected to the tetrahydropterin part of the tricyclic system, the pyranopterin cofactor interfaces with the protein environment through multiple hydrogen bonds. Crystal structures of several Mo enzymes have thus shown that the amino group of pyranopterin forms a hydrogen bond with a nearby cysteine thiolate sulfur atom which is part of an $Fe_nS_4(Cys)_4$ cluster (n = 3 or 4) [13]. This special pterin may thus not only provide a directed electron transfer path but also a switch function for an indirect pathway converting one- and two-electron equivalents similarly as flavins [31]. As a general rule, the necessity for low-energy multielectron catalysis in oxidoreductase enzymes requires "electron-buffer" capacity in the form of either an interaction between several (≥2) metal centers ("clusters") and/or the cooperation between redox-active metal and ligand. While the pterin structure may not be involved in actual oxidation or reduction it can well serve as a mediator for an indirect "superexchange" interaction [33] between the metalladithiolene reaction center and the closest iron-sulfur cluster. In the superexchange model the electron transfer affects the mediator only in a low-lying excited state, taking advantage of high-lying occupied or low-lying unoccupied orbitals of a symmetry appropriate for both connected partner entities. Studies of model systems containing heterocyclic enedithiolates [34] have suggested that there is also a possibility of a thermally induced valence tautomerism of the type (3).

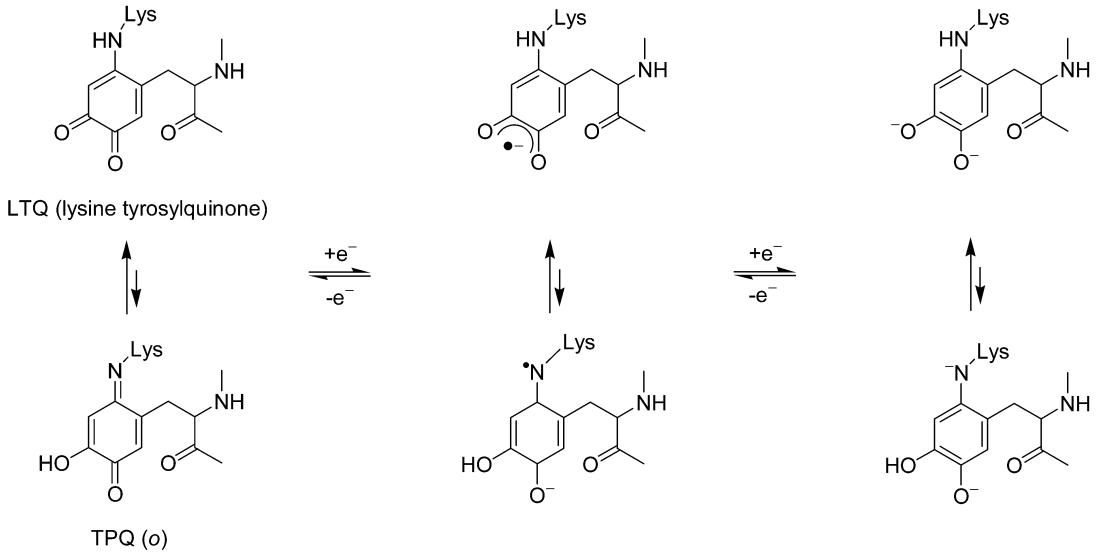
$$[(R_2C_2S_2^{2-})_2\text{–}Mo^{V}(O)]^{-} \longleftrightarrow [(R_2C_2S_2^{2-})(R_2C_2S_2^{\bullet -})\text{–}Mo^{IV}(O)]^{-} \quad (3)$$

AMINE OXIDASES

Copper-dependent amine oxidases are ubiquituous enzymes which catalyse the oxidation of amines to aldehydes (eq. 2) [15–17]. Amine oxidases are typically homodimeric enzymes with one Cu center per subunit. Their biological roles within the general amine metabolism include growth regulation and connective tissue maturation, however, defense functions via H_2O_2 production are also being discussed. Amine oxidases are obvious targets for amine sensor developments. The two-electron oxidation of primary amines to aldehydes is set off by the two-electron reduction of O_2 to H_2O_2 (eq. 2).

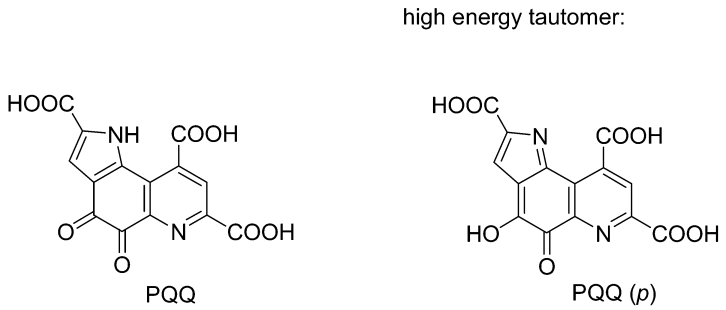
The catalysis of a two-electron process such as (eq. 2) requires a corresponding catalytic site. Since mononuclear biological copper only adopts the oxidation states I and II, the single Type 2 copper center [35] in each subunit requires coupling with a redox-active cofactor, the 2,5-quinone form (topaquinone, TPQ, Scheme 10) of 2,4,5-trihydroxyphenylalanine as covalently linked, post-transla-

tionally modified cofactor [17]. A related monomeric enzyme, lysyl oxidase, uses the similar cofactor lysine tyrosylquinone (LTQ, Scheme 14) [36].



Scheme 14

Metal-dependent quinoproteins are also known with the quinonoid cofactor pyrroloquinolinequinone (PQQ, Scheme 15) [37]. Depending on the substitution pattern, the *o*- (PQQ,LTQ) or *p*-quinone form (TPQ) is favored, however, *o*/*p* tautomeric high-energy species can be conveniently formulated in all instances (Schemes 10, 14, 15). The favored tautomers either avoid the formation of imine instead of carbonyl functions (PQQ, LTQ) or exhibit the established preference for the *p*- over the *o*-tautomer (TPQ).



Scheme 15

Metals such as copper or iron are also essential in the formation and conversion of quinones [38], in other instances, such as the photosystems [8], they may only have a structural role.

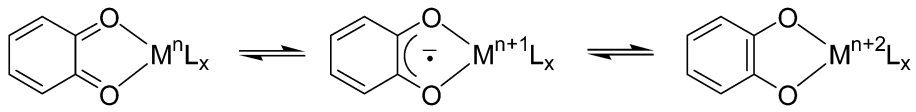
The electronic coupling and mechanistic cooperation between the single copper center and the quinonoid cofactor in amine oxidases is facilitated by the close proximity of the metal and TPQ as evident from structural analysis [35]. Protein crystal structures were reported of copper-dependent amine oxidases from procaryotic and eucaryotic sources. They agree in placing the topaquinone and the metal in close proximity in the active site. In the structure of the enzyme from *E. coli* [35a] an "active" crys-

tal 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 via the oxygen atom in 4-position. In the structure of an enzyme from pea seedling [35b] there is also a loose connection between the metal and the quinonoid ring, suggesting some, probably even essential flexibility in the metal-cofactor interaction. Whereas the triple histidine coordination of the copper center remains a constant feature, the obvious flexibility of TPQ with respect to metal binding is probably essential for enzymatic catalysis [35b]. Extended X-ray absorption fine structure (EXAFS) results of Cu^{I} and Cu^{II} forms confirmed the metal-cofactor interaction [35e].

The requirement for copper may come from the necessity to activate the dioxygen co-substrate in its triplet ground state, $^3O_2$. 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 requires an enzymatically controlled intramolecular electron transfer (4) from the Cu^{II}-catecholate form to the Cu^{I}-semiquinone state.

$$(L)Cu^{I}(Q^{\bullet -}) \rightleftharpoons (L)Cu^{II}(Q^{2-}) \tag{4}$$

Such intramolecular electron transfer (valence tautomer) equilibria involving *ortho*-quinonoid (dioxolene) chelate ligands and transition metals (Scheme 16) have been discovered and studied mainly for manganese, iron, and cobalt complexes [3,39]; only recently have some corresponding copper systems (eq. 4) been reported [40,41].



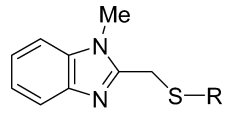
Scheme 16

Equilibrium (eq. 4) has been deduced from EPR spectroscopic studies of substrate-reduced forms of amine oxidases from various sources which 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, i.e., an organic radical species [42]. While the Cu(II) form was recognized as the low-energy state, added cyanide was shown to stabilize the Cu(I)-semiquinone form. Detailed studies of enzyme kinetics confirmed that the Cu(I)-semiquinone state is a viable intermediate [43], probably facilitated through a particular protein-enforced configuration, however, the essentiality of this option and even the metal O_2 binding assumption for enzyme action have been challenged following investigations on metal-substituted, i.e., copper-free analogs [30,44] which, probably facilitated through a particular protein-enforced configuration, were recently shown to have only very low activities [45]. The roles of the metal may include cofactor synthesis, electrostatic activation of the cofactor, and electrostatic stabilization of high-reactivity intermediates such as superoxide [30]; the intramolecular electron-transfer alternative may just provide an additional, more efficient reaction pathway [30,44] as supported also by crystallography of freeze-trapped intermediates of the enzymatic catalysis [35d].

In addition to the perhaps crucial intramolecular electron-transfer step, the overall enzymatic mechanism [17,35] 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 iminoquinone intermediate to the aldehyde and the aromatic form.

There has been a long known dichotomy in the $(Q^{n-})Cu^{+n}L$ complex series, with strong π acceptors L = CO, CNR, ER_3 (E = P,As) favoring the Cu(I)-semiquinone form (n = 1) and conventional (non-π-acceptor) ligands such as amines stabilizing the Cu(II)-catechol state (n = 2) [46]. The observation that thioethers as rather weak π acceptors still favor the Cu(I)-radical state albeit with EPR spectroscopically detectable increased metal contributions [46e] has led to the design of mixed-*N*,*S*-donor lig-

ands such as 1-methyl-2-(alkylthiomethyl)-1*H*-benzimidazoles (Scheme 17) which were found, under the right conditions [40], to yield valence tautomeric complex forms (eq. 4) coexisting in a temperature-dependent equilibrium.



mmb (R = CH_3)

Scheme 17

The capability of 1-methyl-2-(alkylthiomethyl)-1*H*-benzimidazoles and their selenoether analogs (N^E) to tolerate both structurally and electronically quite diverse oxidation states +I and +II of copper has also been demonstrated through DFT-reproduced structural similarities (nearly linear N-Cu-N angle) and fully reversible electrochemical conversion of compounds $[Cu(N\text{^}E)_2]^{+/2+}$, E = S or Se, indicating an unusually small reorganization energy [47]. These systems may thus be viewed as minimal models for Type 1 copper centers [48], combining reversible Cu^I/Cu^{II} conversion with an N_2S_2 donor set [47].

Due to the very different EPR characteristics (g factors, ^{63,65}Cu hyperfine splitting) between Cu^{II} with its $3d^9$ configuration (large g, a_{Cu}) and Cu^I-containing organic π radicals (small g, a_{Cu}) the equilibrium (eq. 4) could be well analyzed using high-resolution EPR at X-band frequency (Fig. 1) [40]. Variation of components Q and L has indicated a remarkable sensitivity of this equilibrium toward perturbation: electron-rich quinones favor the Cu^I-radical form and electron-deficient quinones stabilize the Cu^{II}-catecholate state, with only a few combinations exhibiting a detectable equilibrium situation [40].

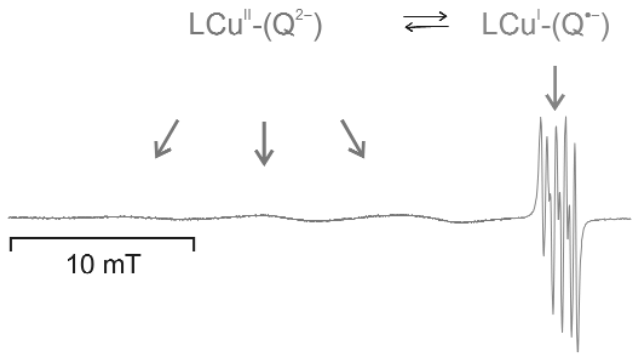


Fig. 1 EPR spectrum of an equilibrium (4) in toluene solution (L = mmb, Q = 3,5-di*tert*butyl-*o*-quinone [40]).

SUMMARY

Cooperation at close distances between one or more redox-active organic molecules and a metal center is more common in biological systems than the existence of the various metallatetrapyrrole chelate compounds suggests. Although direct coordination is rare, a survey of metalloproteins [49] exhibits a number of well-documented cases involving reduced flavins and pterins [22,49], quinones [35,38], and the tyrosyl/tyrosinate pair [8,50,51]. Table 1 shows several such combinations.

Table 1 Examples for metal-ligand cooperation in biochemical systems.

Metal	Ligand	Cofactor or type of protein
Fe	Porphyrin	Heme
Ni	Hydrocorphin	F430
Co	Corrin	Cobalamin
Fe	Flavin	Dehydrogenases
Fe	Pterin	Nitric oxide synthase, aromatic amine acid hydroxylases
Mo, W	Pyranopterin	Oxotransferases
Cu	Quinones	Amine oxidases
Cu, Fe, Mn	Tyrosyl	Galactose oxidase, ribonucleotide reductase, photosystem II

Both the organic and inorganic site may be active for substrate binding and conversion, either through mutual activation or in a separate fashion. While oligonuclear metal clusters appear to be necessary for multielectron-transfer catalysis (Table 2), two-electron processes such as eq. 5 can thus often rely on a single metal center.

Table 2 Representative examples for multielectron catalysis through oligometal active sites in enzymes.

hydrogenases

$$2\,e^- + 2\,H^+ \overset{[Fe_xS_y(Ni)]}{\rightleftharpoons} H_2$$

cytochrome c oxidase

$$4\,e^- + 4\,H^+\,O_2 \overset{\text{Cu/heme}}{\underset{[Mn_4]}{\rightleftharpoons}} 2\,H_2O$$

water oxidase

sulfite reductase

$$6\,e^- + 7\,H^+ + SO_3^{2-} \xrightarrow{[Fe_4S_4]/\text{heme}} HS^- + 3\,H_2O$$

nitrogenase

$$8\,e^- + 10\,H^+ + N_2 \xrightarrow{[Fe_7S_8Mo]} 2\,NH_4^+ + H_2$$

amine oxidase

$$2\,e^- + 2\,H^+ + O_2 \longrightarrow H_2O_2$$

$$RCH_2NH_3^+ + H_2O \longrightarrow RCHO + NH_4^+ + 2\,H^+ + 2\,e^-$$

$$RCH_2NH_3^+ + O_2 + H_2O \xrightarrow{\text{Cu/quinone}} RCHO + H_2O_2 + NH_4^+ \qquad (5)$$

Although the possibility of intramolecular metal/ligand electron transfer to any extent (Scheme 1) is an obvious advantage of such systems, the necessity for such interaction has been questioned in some cases [30,44]; indirect superexchange mechanisms, binding of reactive intermediates, electrostatic effects for cofactor and/or substrate, or even a mere structural role may then justify the requirement of a metal ion.

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