Copper–Dioxygen Chemistry: A Bioinorganic Challenge

ZOLTÁN TYEKLÁR and KENNETH D. KARLIN*

Department of Chemistry, State University of New York (SUNY) at Albany, Albany, New York 12222

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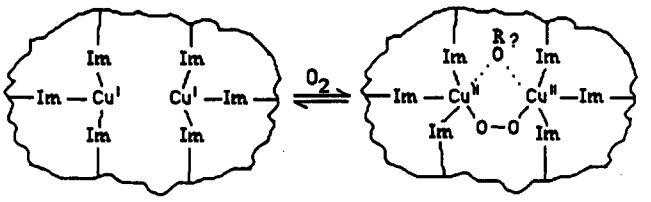
Iron and copper are the predominant metal ion "cofactors" utilized in biological dioxygen (O_2, molecular oxygen) metabolism.[1-3] In the last few years, direct biochemical studies complemented by model investigations have brought about considerable advances in our understanding of (i) heme-iron dioxygen binding in proteins such as myoglobin and hemoglobin[4] and (ii) O_2-activating proteins such as cytochrome P-450 monooxygenase.[5] By contrast, non-heme iron-containing and copper-containing systems are generally less well understood, in spite of the fact that they mediate very similar kinds of chemical processes.

Our own efforts[6] have concentrated on model compounds for O_2-binding or O_2-activating copper proteins. The identification and characterization of relevant coordination complexes and studies of copper complex/O_2/substrate reactions are intended to (a) provide a reasonable basis for hypothesizing biological structures or reaction intermediates, (b) determine the competence of such moieties toward reactivity patterns observed in metalloprotein chemistry, and (c) allow for the future exploitation of copper/O_2 systems as practical dioxygen carriers or as reagents for selective and catalytic oxidative transformations.[3] For the purpose of this Account, our bioinorganic/biomimetic efforts focus on hemocyanins, which bind and transport O_2 in the hemolymph of molluscs and arthropods,[7,8a] and the tyrosinase monooxygenases, which catalyze orthohydroxylation of phenols.[7-9]

Extensive chemical and spectroscopic investigations have contributed to a quite detailed picture of the active sites of hemocyanins[7,8a,10] A recent X-ray structural study on deoxyhemocyanin shows that three imidazole ligands from histidine bind to Cu(I) ions at a dinuclear metal center (Cu···Cu = 3.4–3.7 Å) in a hydrophobic environment that is rich in tryptophan and phenylalanine protein residues.[11] The binding site for copper is rather distorted with each Cu(I) ion having two strongly coordinated imidazole ligands (Cu–N < 2.0 Å) with a third imidazole ligand bound more weakly (Cu–N = 2.4–3.0 Å). No protein-derived ligand serves as a bridging group in deoxyhemocyanin, and the detection of a small group such as OH^- or H_2O would seem to be precluded by the 3.2-Å resolution of the X-ray structure. Oxyhemocyanin is produced by addition of O_2, giving a peroxo (O_2^{2-}) dicopper(II) complex, with Cu···Cu ≅ 3.6 Å the basis of extended X-ray absorption fine structure (EXAFS) measurements.[12] In order to account for this Cu···Cu distance as well as a large body of other spectroscopic and chemical observations, a cis μ-1,2-peroxo moiety along with an additional bridging ligand[13] (probably OH^-) have been proposed for oxyhemocyanin.



deoxy-Hc
Cu...Cu = 3.4 - 3.7 Å

oxy-Hc
Cu...Cu = 3.6 Å

Zoltán Tyeklár was born in Csákvár, Hungary, in 1955. He received his Ph.D. from the University of Veszprém in 1981 for research on copper-catalyzed oxidation reactions of catechols. Following industrial work on triazole-containing pesticides, he performed postdoctoral work (1982–1985) with Professor Gábor Speier at the Institute of Organic Chemistry at the University of Veszprém. He is currently a postdoctoral associate with Professor K. D. Karlin at SUNY Albany. His interests include transition-metal dioxygen chemistry, models for mono- and dioxygenases, spectroscopy and magnetic properties of metal–dioxygen complexes, and interactions of metallic copper with organic substrates.

Kenneth D. Karlin was born in Pasadena, CA, on October 30, 1948. He received his B.S. degree from Stanford University in 1970 and his Ph.D. with S. J. Lippard at Columbia University in 1975. After a term in Cambridge, England, as a NATO postdoctoral fellow with Jack Lewis, he joined the chemistry faculty at the State University of New York (SUNY) at Albany in 1977, where he is now Professor. His research activities center around bioinorganic chemistry with an emphasis on copper, and current pursuits include studies of copper ion redox chemistry, new copper(I) and dinuclear complexes, metal-catalyzed oxidations, and structural and functional modeling of metalloproteins involved in the binding and/or activation of dioxygen. Other interests include metal sulfur-ligand interactions and manganese bioinorganic chemistry.

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(13) In the literature, the additional proposed bridging ligand is often referred to as the "endogenous" bridge, but since a protein derived ligand RO^- donor such as serine or tyrosine appears to be ruled out by the crystal structure on deoxyhemocyanin and sequence analysis studies,[16] OH^- and H_2O are the mostly likely candidates. See ref 14a and: Wilcox, D. E.; Long, J. R.; Solomon, E. I. *J. Am. Chem. Soc.* **1984**, *106*, 2186–2194.

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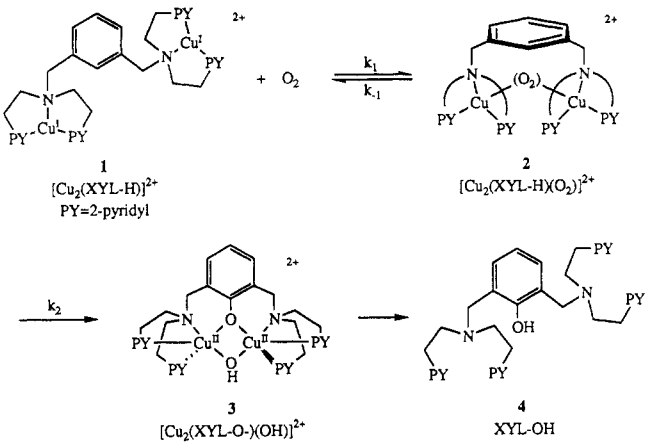

Figure 1. Scheme involving the copper-mediated hydroxylation of an arene. Here, complex **1** reacts with O_2 to give the dioxygen adduct intermediate **2**, which collapses to form the phenoxo-bridged dicopper(II) complex **3**. The free phenol, **4**, can then be isolated by a simple extraction procedure.

Tyrosinases catalyze ortho-hydroxylation of phenolic substrates and further convert the catechols produced to *o*-quinones. As in heme-iron systems, the binding and/or activation of dioxygen appear to be related and associated functions. Detailed investigations by Solomon and co-workers have established the relationship between the active-site chemistry in tyrosinase and hemocyanin, and a mechanism of action has been proposed.[9] An "oxytyrosinase" intermediate with physicochemical properties similar to those of oxyhemocyanin has been characterized, and an important difference between the hemocyanin and tyrosinase active sites is seen to be that in the latter there is an accessibility of phenolic substrate to the dinuclear copper center.

A Deoxyhemocyanin Model and Monooxygenase Reactivity

When we began this work, there seemed to be little prospect for closely modeling the reversible O_2 binding of hemocyanins, although Wilson[14] and Bulkowski et al.[15] had just reported some successes with interesting copper(I) complex systems. We and others had also observed a glaring deficiency of systematic and relevant coordination chemistry of two-, three- or four-coordinate Cu^I or Cu_2^I systems with nitrogen-containing ligands, and so we thought to focus on new structural copper(I) chemistry and associated redox chemistry, utilizing multidentate dinucleating ligands. In this time, a number of other research groups were also studying relevant model dicopper(II) complexes and aspects of Cu(I) coordination chemistry.[16-18]

A dinucleating ligand that has turned out to lead to a wealth of new Cu(I) and associated O_2 chemistry was XYL-H (ligand in structure **1**, Figure 1), where two tridentate PY2 (PY2 = bis[2-(2-pyridylethyl)]amine) donor units are connected by a *m*-xylyl group. XYL-H was fashioned by following the approach of Martell[19] and Bulkowski, et al.[15] Pyridine (PY) donor groups were employed in these model studies in order to mimic the aromatic nitrogen donation from copper protein imidazole groups; the ready synthetic accessibility of PY-containing chelating ligands contributed in large part to their use. Another factor considered was the chemistry already established in using CuCl/pyridine/O_2 or other copper salt/amine systems in effecting a variety of interesting oxidative transformations.[20-22] Tricoordination for Cu(I) seemed most desirable in terms of both stabilizing Cu(I) and then allowing the copper ion to achieve a somewhat typical coordination for the oxidized Cu(II) state upon reaction with and coordination to dioxygen. These ideas came from suggestions outlined by A. D. Zuberbühler which were based on extensive L–Cu(I)/O_2 kinetic studies.[23] Tricoordination for Cu(I) in deoxyhemocyanin had also been suggested by other spectroscopic data,[12b,24] prior to the X-ray structure determination of deoxyhemocyanin.

A dicopper(I) complex containing XYL-H was prepared, and an X-ray crystal structure determination of $[Cu_2(XYL\text{-}H)]^{2+}$ (**1**) confirmed the presence of two well-separated tricoordinate Cu(I) moieties, providing our group's first deoxyhemocyanin model compound (Figure 1).[25] After a number of years of investigations on this and analogous systems, we now know that the reaction of **1** with O_2 does indeed produce a dioxygen/copper adduct (**2**). Furthermore, this reaction also triggers the high-yield hydroxylation of the XYL-H ligand, producing the phenoxo- and hydroxo-bridged dicopper(II) complex **3** (Figure 1).[26] The structure of this complex has also been confirmed by X-ray crystallography (Cu···Cu = 3.1 Å), and isotopic labeling experiments[26] using $^{18}O_2$ confirm that the phenoxo oxygen atoms in **3** and the phenol **4** (derived by leaching out the copper ions from **3**) are derived from molecular oxygen. The observed oxygen atom insertion into an aromatic C–H bond and the stoichiometry of the reaction **1** → **3** (Cu:O_2 = 2:1, obtained manometrically) are characteristics that are reminiscent of the action of the copper monooxygenase tyrosinase. The reaction thus serves as a close model system which can and has provided insights into possible mechanism(s) of copper-mediated dioxygen activation.

By analogy to the formation of O_2 adducts in hemocyanin and tyrosinase, we also presumed that an intermediate dioxygen adduct (peroxo dicopper(II) com-

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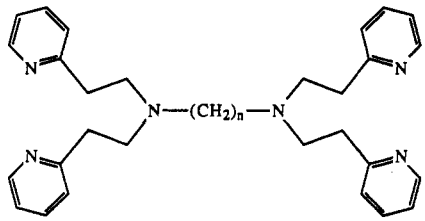
plex?) such as 2 was likely to be formed in the reaction 1 → 3. A hint that this might be true came from the observation that hydrogen peroxide reacts with a dicopper(II) complex of the *m*-XYL-H ligand to produce 3.[27] The presence of two copper ions appears to be necessary to effect the oxygenation, since a mononuclear version of 1 did not give any arene oxygenation when reacted with O_2.[27a,29]

While we have made advances in our understanding of this oxygenation reaction (vide infra), the stereochemical and electronic preferences or requirements are still far from clear. Sorrell has also characterized a complex very similar to 1, having pyrazolyl (pz) donors instead of our pyridine ligands, but the closely related dicopper(I) complex does *not* react with O_2 to provide the analogous hydroxylated ligand.[30] A number of other variations of 1 have been examined (e.g., 6-methylpyridyl groups,[28] mixed ligands containing both py and pz donors,[31] imidazolyl groups,[31b] bis (2-pyridylmethyl)amine instead of PY2 tridentate arms[28]), and these also are found *not* to bring about similar oxygenation chemistry. By contrast, recent studies indicate that several varieties of *m*-xylyl ligands with single "arms" effect ligand hydroxylations upon addition of O_2 to their dicopper(I) complexes.[32]

Evidence for Dioxygen-Copper Complex Intermediates. Analogous Reversible O_2-Binding Systems

In attempting to develop further insights into the mechanism of this important hydroxylation reaction, we chose to synthesize and investigate the chemistry of a range of analogues of complexes of the type 1. One such series of ligands and associated complexes that has also developed into some very exciting new chemistry involves dinucleating ligands with variable-length (*n*) methylene chains connecting the same PY2 tridentate groups used in 1.



Dicopper(I) complexes $[Cu_2(Nn)]^{2+}$, containing two tricoordinate Cu(I) ions, react reversibly with dioxygen at −80 °C in CH_2Cl_2 (Cu:O_2 = 2:1, manometry) to give

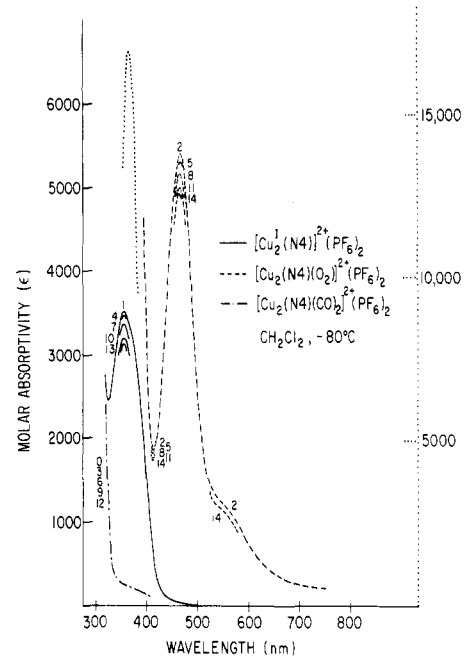


Figure 2. UV–vis spectra demonstrating the reversible O_2 and CO binding behavior of the Cu(I) complexes of N4. The dicarbonyl complex $[Cu_2(N4)(CO)_2]^{2+}$ (featureless spectrum 0) is decarbonylated in vacuo at room temperature, giving the tricoordinate complex $[Cu_2(N4)]^{2+}$ (spectrum 1, λ_{max} 350 nm). Oxygenation at −80 °C generates the dioxygen adduct $[Cu_2(N4)(O_2)]^{2+}$ (spectrum 2, λ_{max} 458 nm). Saturation of this solution with CO followed by partial warming causes the displacement of the O_2 ligand, regenerating $[Cu_2(N4)(CO)_2]^{2+}$ (spectrum 4). The process (*carbonyl cycling*) can be repeated, and five cycles are shown. Cycling experiments involving the direct removal of O_2 from $[Cu_2(N4)(O_2)]^{2+}$ (*vacuum cycling*) can also be followed spectrophotometrically.[38] The strong 360-nm band of $[Cu_2(N4)(O_2)]^{2+}$ (···) is also shown.

O_2 adducts formulated as $[Cu_2(Nn)(O_2)]^{2+}$.[6,33] The UV–vis spectral features observed for these species are unique for copper coordination complexes and in Cu(I)/O_2 chemistry. They are characterized by multiple and strong charge-transfer bands in the 330–600-nm region, which resemble to a great extent the UV–vis spectrum observed for oxyhemocyanin. Complexes $[Cu_2(Nn)(O_2)]^{2+}$ are stable at reduced temperatures only, as indicated by the complete loss of these spectral bands upon warming. Observation of d–d bands at >650 nm and results from X-ray absorption edge studies[34] suggest that Cu(II) is present. Thus, the O_2 adducts are best formulated as peroxo–dicopper(II) complexes.

The bound dioxygen (peroxo) ligand in $[Cu_2(Nn)(O_2)]^{2+}$ can be removed by the application of a vacuum, or it can be displaced by carbon monoxide forming the biscarbonyl adduct, $[Cu_2(Nn)(CO)_2]^{2+}$. This latter process is suggested to occur by shifting the dioxygen binding equilibrium toward the deoxy form, $[Cu_2(Nn)]^{2+}$, with subsequent reaction with CO. Cycling experiments, demonstrating the *reversible binding of CO and O_2* to complexes $[Cu_2(Nn)]^{2+}$, can be followed spectrophotometrically, and Figure 2 illustrates this for the N4 system.[33]

(27) However, the mechanism of this reaction with H_2O_2 has been shown to be different than that observed for the Cu_2^I/O_2 reaction. (a) Blackburn, N. J.; Karlin, K. D.; Concannon, M.; Hayes, J. C.; Gultneh, Y.; Zubieta, J. *J. Chem. Soc., Chem. Commun.* **1984**, 939–940. (b) Cruse, R. W.; Kaderli, S.; Meyer, C. J.; Zuberbühler, A. D.; Karlin, K. D. *J. Am. Chem. Soc.* **1988**, *110*, 5020–5024.

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(29) Low-temperature oxygenation of a mononuclear Cu(I) complex containing an N-benzylated PY2 group[27a] does generate a dioxygen adduct, and some ligand oxygenation occurs, giving benzaldehyde.[28]

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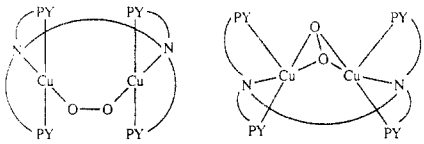
The N*n* ligands contain amine donors only and, thus, no obvious monoatomic bridging group for the copper ions (e.g, Cu–X–Cu). The results indicate that a bridging ligand besides O_2^{2-} itself is *not* a prerequisite for systems capable of binding CO and O_2 reversibly, nor for generating spectral characteristics reminiscent of oxyhemocyanin. Thus, complexes $[Cu_2(Nn)(O_2)]^{2+}$ possess magnetically coupled Cu(II) centers (i.e., EPR silent, sharp ^1H NMR spectra) with a peroxo bridging group possessing a binding mode that is capable of mediating strong antiferromagnetic coupling. Direct magnetic susceptibility or other suitable measurements will be necessary to quantitate and confirm this assertion. At least subtle structural differences among complexes $[Cu_2(Nn)(O_2)]^{2+}$ are apparent, based on the noticeable UV–vis spectral differences seen for variations in *n* of the ligand N*n*. Solution EXAFS studies of frozen solutions of $[Cu_2(Nn)(O_2)]^{2+}$ suggest that the Cu···Cu distance varies between 3.2 and 3.4 Å for the complexes with $n = 3$ or 4.[34] The peroxo binding mode is uncertain at this time, but on the basis of the X-ray absorption study and other considerations, we have suggested either a μ-1,2- or possibly a μ-η^2:η^2-peroxo coordination mode in these complexes.[33,34] The latter



Cu...Cu = 3.2 - 3.4 Å (EXAFS)

has not previously been considered for O_2 binding to copper in hemocyanin or in complexes.

The obvious close relationship between the ligands in the dioxygen complexes $[Cu_2(Nn)(O_2)]^{2+}$ and XYL-H (Figure 1) appears to support our supposition that a dioxygen complex (**2**) is likely to be involved as an intermediate in the hydroxylation reaction **1** → **3**. To provide further evidence for this hypothesis, we synthesized the ligand XYL-F, in which a fluorine atom was placed in the 2-position of the XYL-H ligand, potentially blocking the position that is hydroxylated.[6,35] Indeed, oxygenation (–80 °C in CH_2Cl_2) of the derived dicopper(I) complex, $[Cu_2(XYL\text{-}F)]^{2+}$, provides a *stable* complex formulated as $[Cu_2(XYL\text{-}F)(O_2)]^{2+}$ (Cu:O_2 = 2:1) and renders a UV–vis spectrum very similar to that of $[Cu_2(N5)(O_2)]^{2+}$. We note that N5 seems most closely related to the XYL-H ligand in complex **1**, since it has a five-carbon chain connecting PY2 units, and a comparison of UV–vis data for complexes $[Cu_2(N5)(O_2)]^{2+}$ and $[Cu_2(XYL\text{-}F)(O_2)]^{2+}$ bears this out.[6,35] At –100 °C in CH_2Cl_2, oxygenation of the parent XYL-H containing complex itself, $[Cu_2(XYL\text{-}H)]^{2+}$ (**1**), causes the appearance of a metastable spectrum similar to that observed for complexes $[Cu_2(N5)(O_2)]^{2+}$ and $[Cu_2(XYL\text{-}F)(O_2)]^{2+}$,[35] attesting to the intermediacy of a dioxygen complex $[Cu_2(XYL\text{-}H)(O_2)]^{2+}$ (**2**) (Figure 1).

In a recent collaborative kinetic/spectrophotometric study with A. D. Zuberbühler and co-workers, evidence for the intermediate **2** has been obtained.[36] In this this *m*-XYL-H hydroxylating system, the data analyses indicate that **1** reacts reversibly with O_2 to form a dioxygen adduct **2** (characterized by the strong absorption at 435 nm), which then irreversibly "decomposes" in a first-order process providing the product **3** (Figure 1). This investigation furnishes the first kinetic and thermodynamic parameters for reversible binding of dioxygen to copper coordination complexes, with $k_1 = 533$ M^{-1} s^{-1} and K_{eq} (k_1/k_{-1}) = 2.7×10^6 at –80 °C, $\Delta H° = -62 \pm 1$ kJ mol^{-1}, $\Delta S° = -200 \pm 6$ J mol^{-1} K^{-1}, $\Delta H_1^{\ddagger} = 8.15 \pm 0.07$ kJ mol^{-1}, $\Delta S_1^{\ddagger} = -146.9 \pm 0.3$ J mol^{-1} K^{-1}. It is also interesting to note that k_2 appears to be unaffected by substituting the hydrogen atom with deuterium in the 2-position of the ligand.

Possible Mechanism for the Copper Monooxygenase Model System

A particularly interesting and possibly important insight into the *m*-xylyl hydroxylation mechanism came in recent experiments in which a methyl group was placed into the 2-position of the ligand, where hydroxylation normally occurs. Instead of causing benzylic hydroxylation or perhaps blocking any oxygenation reaction of the aromatic ring as was the case for 2-fluoro substitution, hydroxylation at the 2-position and migration of the methyl group took place. This reaction appears to be the first example of a copper-mediated hydroxylation-induced alkyl group migration.[6b,37]

The process is reminiscent of the "N.I.H. shift", where electrophilic attack of a presumed metal–oxy (dioxygen derived) species results in hydroxylation-induced migrations of groups such as ^2H, ^3H, halogen, and methyl in substrates for the non-heme iron dependent phenylalanine hydroxylases as well as in cytochrome P-450 monooxygenases.[6b,37] For reaction of **5** with dioxygen, one can thus propose a mechanism[37] involving an electrophilic dioxygen complex intermediate (**6**) that attacks the arene ligand. This is followed by 1,2-migration of the methyl group, rearomatization with "assistance" of the aliphatic amine nitrogen lone pair, and loss of the iminium ion, $[[(PY)CH_2CH_2]_2N{=}CH_2]^+$ (PY = 2-pyridyl), in a retro-Mannich reaction. Under the experimental conditions employed, PY2 and formaldehyde are the observed products along with the dimeric Cu(II) complex containing the phenol product (**7**, Figure 3). As such an "N.I.H shift" mechanism is applied to the reaction of the parent complex $[Cu_2(XYL\text{-}H)]^{2+}$ (**1**) with O_2, the 2-H proton would be the migrating group, and during rearomatization, H^+ can be easily lost in preference to the ligand arm. The lack of a 2-deuterio isotope rate effect (vide supra) is also consistent with the proposed mechanism.

The results here thus point to arene hydroxylation by an electrophilic intermediate, probably the Cu_2–O_2 complex described above, or a copper/oxygen species derived from it. This undoubtedly forms in close and appropriate proximity to the xylyl group of the dinucleating ligand (XYL-R). The μ-1,2- or μ-η^2:η^2-peroxo dicopper (II) complex structure we have suggested for complexes $[Cu_2(XYL\text{-}H)(O_2)]^{2+}$ (**2**) and $[Cu_2(Nn)(O_2)]^{2+}$ could be electrophilic in nature. In this connection, we observe that the dioxygen (peroxo) ligands in complexes $[Cu_2(Nn)(O_2)]^{2+}$ are not readily protonated, and they appear to behave much like peroxo complexes of early

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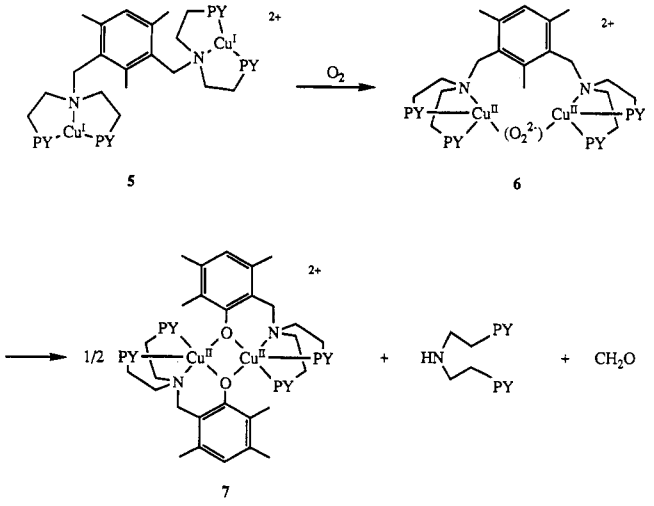


Figure 3. Oxygenation of dicopper(I) complex **5**, which results in the formation of **7**, where the 2-methyl group of the ligand has migrated, and loss of a PY2 ligand arm and formation of formaldehyde has occurred. The scheme bears analogy to the "N.I.H. shift" mechanism proposed for iron hydroxylases and suggests that an intermediate such as **6** is capable of electrophilic attack of the aromatic substrate.

transition metals.[38] By contrast, in the two other types of Cu_2–O_2 complexes we have characterized (vide infra), protonation readily gives hydrogen peroxide, and the bound dioxygen (peroxo) ligand appears nucleophilic, as judged by its reactions toward substrates such as SO_2 and CO_2.[38]

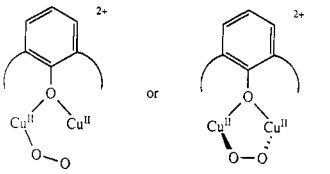
As it may pertain to the monooxygenases tyrosinase and the copper phenylalanine hydroxylase,[39] the results here reinforce the previous suggestions[9,40] that hydroxylation in these enzymes may proceed via electrophilic attack on the aromatic substrates.

A Phenoxo-Bridged Reversible O_2-Binding System. Peroxo and Hydroperoxo Dicopper(II) Complexes

We have found that the phenol, XYL-OH (**4**), produced by the hydroxylation reaction **1** → **3** (Figure 1) provides for a great deal of interesting coordination chemistry, including a complex that can be used to bind dioxygen reversibly and also to produce a hydroperoxo dicopper(II) complex.

Thus, XYL-OH (**4**) can be used to synthesize the dinuclear phenoxo-bridged copper(I) complex, $[Cu_2(XYL\text{-}O\text{-})]^{2+}$ (**8**), that binds O_2 as well as CO reversibly.[41] An X-ray structure of **8** shows that it possesses some features that are strikingly similar to those of the proposed sites of either deoxy- and/or oxyhemocyanin, including the Cu···Cu distance of 3.6–3.7 Å, an "endogenous" phenoxo bridging group, and an empty "pocket" where a second small bridging group (X), such as OH^- (e.g., **3**), N_3^-, Cl^-, Br^-, and RCO_2^-[41] is known to coordinate in dicopper(II) complexes $[Cu^{II}_2(XYL\text{-}O\text{-})(X)]^{2+}$.

When an orange dichloromethane solution of **8** is exposed to dioxygen below −50 °C, an intense purple color develops due to the formation of the dioxygen adduct **9** (Figure 4). Manometric measurements at −80 °C indicate that 1 mol of dioxygen is taken up per mole of **8** to give the product formulated as **9**, $[Cu^{II}_2(XYL\text{-}O\text{-})(O_2^{2-})]^+$. A detailed resonance Raman study carried out by Professor E. I. Solomon's group at Stanford University revealed that the dioxygen (peroxo, O_2^{2-}, $\nu_{O\text{-}O}$ = 803 cm^{-1}) ligand is unsymmetrically bound.[42] EXAFS results have indicated a 3.31-Å copper–copper separation for **9**.[43] This finding rules out a μ-1,1-bridging peroxo geometry for $[Cu^{II}_2(XYL\text{-}O\text{-})(O_2^{2-})]^+$, since structural data for the doubly bridged complexes $[Cu^{II}_2(XYL\text{-}O\text{-})(X)]^{2+}$ show that a μ-1,1-X (X = oxygen atom) bridging geometry is only compatible with a Cu···Cu distance of <3.15 Å. Thus, the structural information and spectroscopic analysis are explained by the presence of a terminally bound peroxo unit, i.e., $Cu^{II}(O_2^{2-})$, and so **9** possesses either a nonsymmetrical μ-1,2-bridging peroxo ligand (e.g., axial to one Cu, but equatorial to the other) or it is bound to a single Cu^{II} ion.[42]



Cu...Cu = 3.31 Å (EXAFS)
O_2^{2-} unsymmetrically bound

The kinetics and thermodynamics for the reversible binding reaction of $[Cu_2(XYL\text{-}O\text{-})]^{2+}$ (**8**) with O_2 have also been examined, and $k_{on} > 10^6$ M^{-1} s^{-1} and K_{eq} (k_{on}/k_{off}) = 7.3×10^7 at −80 °C; $\Delta H° = -66.2 \pm 0.5$ kJ mol^{-1}, $\Delta S° = -192 \pm 2$ J mol^{-1} K^{-1}.[36] A comparison with the data on the oxygenation of $[Cu_2(XYL\text{-}H)]^{2+}$ (**1**) indicates a considerably faster O_2 binding rate of reaction (i.e., k_{on}) for **8**. This may be due to (i) the effect of forcing the two Cu(I) ions into close proximity by the presence of a bridging phenoxo ligand in **8** and/or (ii) the likely greater ease of oxidizing Cu(I) in **8** compared to **1**, due to the presence of the "hard" phenoxo oxygen ligand in the former.

The application of a vacuum while rapidly warming solutions of **9** results in the removal of O_2, and regeneration of dicopper(I) complex **8** and quasi-reversible cycling between **8** and **9** using such vacuum-purge applications can be followed spectrophotometrically.[41] Dioxygen can also be liberated by addition of carbon monoxide or triphenylphosphine to the purple solution of **9**: the purple color fades and the bis(carbonyl)dicopper(I) complex **10a** or the bis(triphenylphosphine) adduct **10b** ($[Cu^I_2(XYL\text{-}O\text{-})(L)_2]^+$, **10**, L = CO, PPh_3) is formed. Carbon monoxide can be removed from $[Cu^I_2(XYL\text{-}O\text{-})(CO_2)]^+$ (**10a**) by applying a vacuum to give back $[Cu^I_2(XYL\text{-}O\text{-})]^+$ (**8**), and several cycles of oxygenation, dioxygen displacement by CO, and decarbonylation can be carried out without a severe amount of decomposition (Figure 4).

The observation that the peroxo dicopper(II) complex $[Cu^{II}_2(XYL\text{-}O\text{-})(O_2^{2-})]^+$ (**9**) does not oxygenate a substrate as readily oxidizable as PPh_3 is of interest, par-

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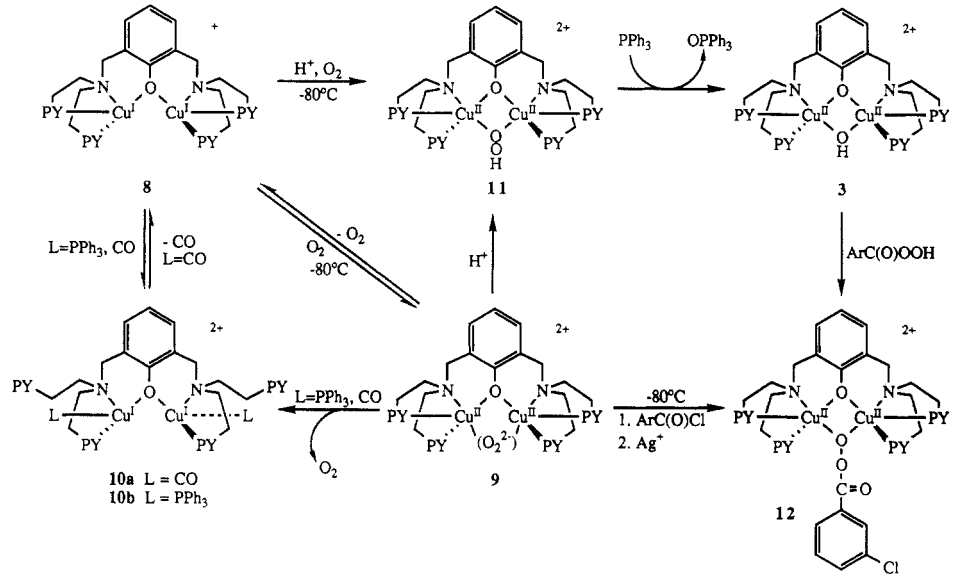

Figure 4. Scheme describing the complexes of the phenol ligand XYL-OH. Complex **8** is a reversible CO and O_2 carrier, and the dioxygen [peroxo–dicopper(II)] complex **9** can be protonated to give a hydroperoxo complex **11** or acylated to give compound **12**. Both of the latter [Cu_2(XYL-O-)(OOR$^-$)] complexes react with PPh_3 to give O=PPh_3 and **3**, but no oxidation of PPh_3 occurs upon its reaction with the peroxo complex **9**. Instead, O_2 is liberated and the PPh_3 adduct (**10b**) forms.

ticularly when contrasted with the corresponding reaction with a protonated form of **9**, the hydroperoxo complex [Cu^{II}_2(XYL-O-)(OOH)$^-$)]$^{2+}$ (**11**) (Figure 4).[44] Complex **11** is suggested to have a μ-1,1-OOH$^-$ structure, as shown, since a solution EXAFS study indicates a structure similar to [Cu^{II}_2(XYL-O-)(OH$^-$)]$^{2+}$ (**3**) and the Cu^{II}···Cu^{II} distance is ~3.04 Å. Strong evidence for this structure also comes from the similarity of spectral properties and reactivity of **11** with the X-ray structurally characterized acylperoxo complex [Cu^{II}_2(XYL-O-)(*m*-$ClC_6H_4CO_3^-$)]$^{2+}$ (**12**), which can be formed by the acylation of the dioxygen complex **9** (Figure 4).[6b,45] Both [Cu^{II}_2(XYL-O-)(OOR$^-$)]$^{2+}$ [R = H or *m*-ClC_6H_4C(O)] complexes react quantitatively with PPh_3 to give O=PPh_3 plus [Cu^{II}_2(XYL-O-)(OR$^-$)]$^{2+}$ (**3**, R = H).

These observations are in accord with results obtained for other transition metal peroxide complexes where the oxidation of organic substrates is enhanced by the presence of electrophiles such as H^+ or RC(O)$^+$.[3,6b,45] In the present case, the protonation (or acylation) of the dioxygen–copper complex appears to result in activation, via formation of a Cu_n–OOR species capable of transferring an oxygen atom to a substrate, while leaving behind a stable hydroxo (or carboxylato) Cu^{II}_n moiety.

The possible biological relevance here pertains to the activation of dioxygen in copper monooxygenases where protonation of a Cu^{II}_n (n = 1 or 2) bound peroxo ligand derived from reaction of $2Cu^I + O_2$ may lead to O–O "degradation" processes in the presence of substrates, resulting in oxygen atom transfer reactions. Thus, we can speculate that in tyrosinase, proton transfer (from phenol substrate?) to the Cu–(O_2)–Cu moiety provides a Cu^{II}_n–OOH activated species, which effects the hydroxylation of the aromatic substrate (by electrophilic attack, vide supra). Without consideration of the

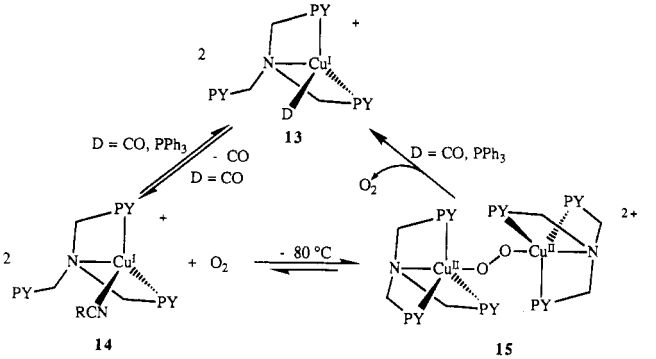

Figure 5. Reversible binding of O_2 and CO by copper complexes of tris(2-pyridylmethyl)amine (L). This ligand chelates as a tridentate donor in mononuclear Cu(I) complexes along with a fourth donor group such as RCN (**14**), CO, or PPh_3 (**13**). The reaction of O_2 with **14** provides a dinuclear peroxo-bridged dicopper(II) complex (**15**), which has been crystallographically characterized.

possible role of protons, Solomon and co-workers[9] have previously suggested that the binding of phenol substrate to one of the Cu(II) ions in the Cu–(O_2)–Cu intermediate causes a labilization of the bound peroxo ligand resulting in its polarization and activation toward attack of the proximate substrate. A hydroperoxo species may also be important in dopamine β-hydroxylase, and Klinman and co-workers[46] have proposed that a mononuclear {Cu^{II}–OOH}$^+$ moiety is the active oxidant in the dopamine β-hydroxylase catalyzed benzylic hydroxylation reaction. This species may be formed from a $2Cu^I/O_2$ reaction with a protein-derived acid group supplying a proton to peroxide which is then bound at a single copper ion site.

Crystal Structure of a LCu–O_2–CuL Complex

While structural information exists for the binding of dioxygen to heme iron as well as mono- and dinuclear cobalt coordination complexes,[2-4,47] the detailed crys-

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tallographic characterization of a Cu_n–O_2 species has for years been an elusive goal of researchers investigating copper/dioxygen reactivity. Recently, we have been able to achieve this objective, in a system where the copper(I) precursor also binds the dioxygen ligand reversibly.[48]

The tripodal tetradentate ligand tris(2-pyridylmethyl)amine (L) can be used to form cationic copper(I) complexes $[LCu(D)]^+$ (**13**, D = RCN, CO, PPh_3) (Figure 5). In CH_3CH_2CN (EtCN) solvent, reaction of the orange compound $[LCu(RCN)]^+$ (**14**) with dioxygen at −80 °C ($Cu:O_2$ = 2:1, manometry) produces an intensely purple colored complex, $[\{LCu\}_2(O_2)]^{2+}$ (**15**), which is EPR silent and is characterized by strong UV–vis absorptions at 525 (ϵ = 11 500 M^{-1} cm^{-1}) and 590 (sh, ϵ = 7600) nm (probably $O_2^{2-} \rightarrow$ Cu(II) LMCT transitions) with an additional band at 1035 nm (ϵ = 180).

As with the other dioxygen–copper complexes described above, the binding of O_2 (and CO) to $[LCu(RCN)]^+$ (**14**) is reversible, as evidenced by the reactions interconverting complexes **13**–**15** (Figure 5). (i) When a vacuum is applied to $[\{LCu\}_2(O_2)]^{2+}$ (**15**) in EtCN while heating briefly, the purple solution decolorizes and $[LCu(EtCN)]^+$ (**14**) is produced. Rechilling (<−80 °C) followed by introduction of O_2 regenerates **15**, and this procedure can be repeated several times without severe decomposition. (ii) Dioxygen can be displaced from **15** by reaction with either PPh_3 or CO (EtCN) to give the adducts $[LCu(D)]^+$ (**13**). In both cases, O_2 is detected qualitatively by passing the gas produced through a test solution of alkaline pyrogallol, and for PPh_3, evolution of O_2 (>80%) is observed by manometry. (iii) Carbon monoxide can be used to effect repetitive "CO cycling" where, in EtCN, O_2 is displaced from $[(LCu]_2(O_2)]^{2+}$ (**15**) giving $[LCu(CO)]^+$ (**13**, D = CO), the CO is removed via vacuum/Ar-purge cycles (room temperature) providing $[LCu^I(RCN)]^+$ (**14**), and rechilling of **14** followed by oxygenation regenerates **15** (Figure 5).

Thermally and hydrolytically unstable crystals of $[\{LCu\}_2(O_2)](PF_6)_2 \cdot 5Et_2O$ (**15**-$(PF_6)_2 \cdot 5Et_2O$), were obtained at −85 °C in EtCN, and X-ray data were obtained at −90 °C. The complex is best described as a peroxo dicopper(II) species, and as shown in Figure 6, it contains a trans μ-1,2-O_2^{2-} group (derived from O_2) bridging the two Cu(II) ions. As found in other Cu(II) complexes with L, the Cu atom is pentacoordinate with a distorted trigonal bipyramidal geometry, and the peroxo oxygen (O1) atoms occupy axial sites. The Cu–Cu′ separation is 4.359 (1) Å, and the O1–O1′ bond length is 1.432 (6) Å, which are structural parameters similarly found for peroxo-bridged dicobalt(III) complexes.

This structural determination of a Cu_2–O_2 complex conclusively demonstrates that such species exist and that the difficulties inherent in their characterization (e.g., Cu(I) disproportionation, kinetic lability of Cu(I) and Cu(II), and copper-catalyzed peroxide decomposition) can be overcome. In light of the considerable efforts put forth to generate and describe Cu_n–O_2 complexes based on the known or presumed properties of the active site in hemocyanins, it is interesting to note

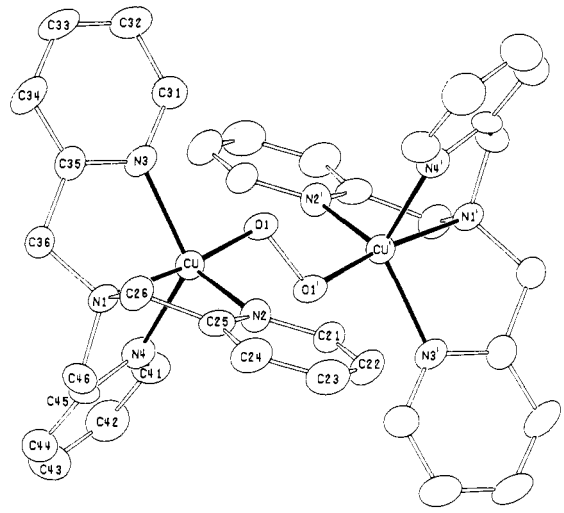

Figure 6. ORTEP diagram of the peroxo–dicopper(II) complex $[\{LCu\}_2(O_2)]^{2+}$ (**15**), based on the X-ray structural determination carried out at −90 °C.

that the present case utilizes *mononuclear* Cu(I) complexes to bind O_2, and that *no* additional bridging ligand (i.e., besides O_2^{2-}) such as RO^- is needed. The coordination geometry in $[\{LCu\}_2(O_2)]^{2+}$ (**15**) found for Cu(II) is, in fact, rather ordinary, and the binding properties and structure parallel those for the well-established peroxo dicobalt(III) compounds. Compound **15** is *not* a good structural model for the active site of oxyhemocyanin, but the complex will allow us to assess the relatively unexplored reactivity patterns and magnetic and spectroscopic contributions of an O_2^{2-} ligand bound to copper.

Conclusions

Synthetically derived copper–dioxygen coordination complexes do indeed exist. Reversible O_2-binding systems are known, and structural characterization of Cu_2–O_2 complexes is possible.

In our own laboratories, the key success elements seem to have included the use of low-temperature experimental approaches and the fortunate choice of donor groups and ligand design for mono- or dinuclear copper complexes. At least for the types of ligands we have employed, the stability and "visibility" of copper–dioxygen complexes seems due to the rapid L-Cu_2^I–O_2 "on" rates at low temperatures, relative to the rates of the subsequent decomposition reactions. The latter consist of the internal hydroxylation in the conversion **1** → **3**, disproportionation processes where the stoichiometry of reaction is $Cu:O_2$ = 4:1 and the products are hydroxo or oxo copper(II) compounds, or direct reaction with solvent and/or water.[6,17b,41,49]

Here, we have described three different types of Cu_2–O_2 complexes in which O_2 (and CO) binding to Cu(I) precursors is reversible. Others have also been described;[6,18b] due to their UV–vis and/or IR/resonance-Raman spectroscopic properties, the most interesting appear to be the reversibly O_2 bound LCu–O_2 (Cu^{II}-superoxo)[50] and peroxo compounds of Thompson[51]

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and the recently reported peroxo complex of Kitajima et al.[52] Undoubtedly, other new systems will be characterized, and important goals will be to develop an understanding of structure/spectroscopic and reactivity correlations for Cu_n–O_2 complexes. The acquisition of basic kinetic/thermodynamic data as it relates to known structural type is also an important aspect.

The development of new and improved copper–dioxygen complex systems is desirable, including those with improved thermal stability compared to the ones described in this Account. More accurate models for the active site in deoxy- and oxyhemocyanin are needed. While complexes $[Cu_2(Nn)(O_2)]^{2+}$ possess spectroscopic features similar to those of oxyhemocyanin, there are differences, and the Cu···Cu distances in these solution species do not duplicate that found for the protein. The identity, spectroscopic effect, and coordination role of a bridging ligand besides O_2^{2-} for the Cu_2–O_2 unit in oxyhemocyanin and/or model complexes are not settled. From the monooxygenase model system described herein, we feel that we have developed certain mechanistic insights concerning chemical and biological copper-mediated dioxygen activation. Additional approaches are clearly needed to help understand reaction mechanism(s) in both copper oxygenases and oxidases. Here, the understanding of Cu_n–O_2 and/or Cu_n–OOH reactivity and O–O bond cleavage processes would appear to be crucial.

The crystallization and X-ray structural determination of a Cu–O_2–Cu complex for the first time will allow the direct assessment of the magnetic and spectroscopic behavior and the reactivity of a peroxo (O_2^{2-}) ligand with known ligation; it will also provide a framework for the design of new systems. We feel that copper/dioxygen coordination and bioinorganic chemistry is just in its infancy.

We thank Ninian Blackburn, Ed Solomon, Andreas Zuberbühler, and Jon Zubieta and their associates for their collaborative efforts and substantial contributions to the research described here. The dedicated research efforts and contributions of the undergraduate and graduate students and postdoctorals at Albany are also acknowledged; their names are listed in the relevant references. We also are very grateful to the National Institutes of Health for the support of the research described here.

Registry No. O_2, 7782-44-7; Cu, 7440-50-8; tyrosinase, 9002-10-2.

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