CATECHOL OXIDASE AND BIOMIMETIC APPROACHES

Krebs, B.; Merkel, M.; Rompel, A.

Institut für Anorganische und Analytische Chemie, Westfälische Wilhelms-Universität, Wilhelm-Klemm-Strasse 8, 48149 Münster, Germany
Phone: +49 (251) 8333131, Fax: +49 (251) 8338366, E-mail: krebs@uni-muenster.de

Received February 27th, 2004. In final form, March 3rd, 2004.
Dedicated to Prof. Dr. Pedro A. Aymonino on the occasion of his 75th birthday.

Abstract

Transport, activation, and metabolism of dioxygen are very important processes in many living organisms. These functions are often assigned to metalloproteins containing iron or copper. Especially the iron containing heme proteins present the most well-known examples like hemoglobin and myoglobin (oxygen transport), cytochrome c oxidase (activation of dioxygen and oxidation) or peroxidase (elimination of radical intermediates). Apart from heme species metalloproteins with a dinuclear metal center containing iron or copper serve as biological alternatives. Examples for oxygen transport are hemerythrin containing iron and hemocyanin containing copper. These dinuclear centers also appear in proteins with enzymatic activity. The copper containing species are called copper-type 3 proteins.

Resumen

Este minireview describe la estructura y mecanismos de las catecol oxidasas (COs). Estas enzimas son aisladas de diferentes fuentes vegetales, existiendo información estructural, obtenida por difracción de rayos X, para la CO de batatas. Se presentan compuestos modelo relevantes, con un cierto énfasis en peroxo complejos de cobre, considerándose sus características de enlace y comentando sus diferencias básicas con los sistemas relacionados tirosinasa y hemocianina, que son también proteínas conteniendo cobres de tipo 3.

Catechol oxidase

Catechol oxidases (CO), E.C. 1.10.3.1, represent a group of ubiquitous oxidases in plants. Catechol oxidase, tyrosinase (TYR), E.C. 1.14.18.1 and E.C. 1.10.3.1, and hemocyanin (HC) belong to the class of copper-type 3 proteins. Their functions are dioxygen transport (HC) and oxygen activation (CO and TYR). CO catalyzes exclusively the oxidation of catechols (i.e. *ortho*-diphenols) to the corresponding *ortho*-quinones (catecholase activity). In contrast to CO the related TYR reveals, in addition to catecholase activity, a monooxygenase activity (cresolase activity, E.C. 1.14.18.1) that enables the enzyme to accept monophenols, e.g. tyrosine, as substrate (see Figure 1). [1] HC is the oxygen transport protein in many molluscs and arthropods. [2,3]

CO was first isolated by Kubowitz in 1937 from potatoes. [4,5] Meanwhile COs have been purified from different sources among those are potatoes, spinach, apple, grape berry [6]

lychee fruit[7] beans[8], banana[9], opium plant [10] coffee plant[11], black poplar[12] and gypsy wort [12]. The existence of isozymes is a major problem, that occurs during purification of CO and TYR. [13,14]

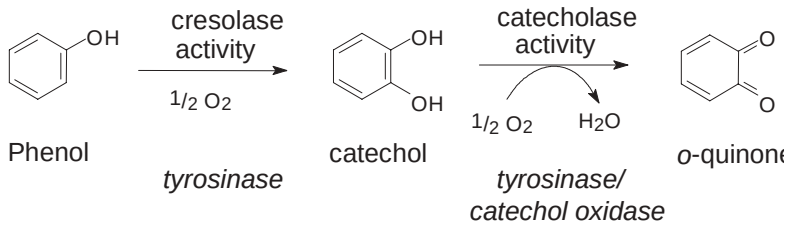

Figure 1: *Cresolase activity and catechol activity of tyrosinase and catechol oxidase*

For all type 3 copper proteins the dicopper center is spectroscopically well characterized by UV/Vis, [15, 16] EPR, [17,18] NMR, [19] EXAFS, [20,21,22,23] magnetic, and Resonance Raman [24,25] studies. The *met* form of CO contains two Cu(II) ions and is EPR silent due to strong antiferromagnetic coupling between the two S = 1/2 copper ions. The antiferromagnetic coupling requires a superexchange pathway associated with a bridging ligand which is most likely a μ-hydroxo group bridging the two Cu ions. *Oxy* CO can be obtained by adding H_2O_2 to *met* CO or addition of oxygen to the *deoxy* Cu(I)-Cu(I) form. Evidence for the oxygen binding mode can be obtained from UV/Vis data in combination with Resonance Raman data. *Oxy* CO shows two characteristic peroxo → Cu(II) *charge transfer* transitions. The absorption band at 345 nm is caused by an O_2^{2-} (π^*_σ) → Cu(II) ($d_{x^2-y^2}$) *charge transfer* transition. The absorption band at 580 nm corresponds to the second O_2^{2-}(π^*v) → Cu(II) ($d_{x^2-y^2}$) *charge transfer* transition. The Resonance Raman spectrum of the *oxy* form shows a band ~ 750 cm^{-1} characteristic for a μ-η^2:η^2 binding mode for dioxygen first reported by Kitajima et al. [26] for a synthetic binuclear copper model complex. EXAFS studies of *Neurospora crassa* TYR and sweet potato CO revealed a Cu(II)-Cu(II) distance of 3.6 Å and 3.8 Å, respectively for the *oxy* species and 3.4 Å and 2.9 Å, respectively for the *met* form. [27]

^{1}H NMR spectra recorded for TYR (*Streptomyces antibioticus)* confirm that both copper atoms are coordinated by N_ε atoms of histidine ligands. There is no evidence that the bridging ligand arises from an amino acid residue. [19] Furthermore the binding of chloride to TYR could be shown. [19] It is under discussion that chloride bridges the two copper ions in μ_2 fashion, as it has been suggested for the *half met* Form (Cu(I)-Cu(II)) of TYR from (*Neurospora crassa)* and the molluscan HC. [17,21,28]

For several years only two arthropodan HC crystal structures were available, spiny lobster (*Panulirus interruptus*) [29] and horseshoe crab (*Limulus polyphemus*). [30] In 1998, a molluscan HC from giant octopus (*Octopus dofleini*) was crystallized and its three-dimensional structure determined. [31] Also in 1998 Krebs' group succeeded in obtaining CO crystals from sweet potato (*Ipomoea batatas*, ibCO) for 2.5 Å structure determination of the first type 3 copper enzyme. [32] Up to now a protein structure of TYR is not available.

The crystal structure of a plant CO containing a dinuclear copper center

Overall structure and folding of ibCO

Monomeric ibCO, with a molecular mass of 39 kDa, is ellipsoid in shape with dimensions of 55 * 45 * 45 Å. The secondary structure is dominated by α-helical and coiled regions. The structure shows a four helix bundle surrounding the dinuclear copper center as the most striking structural motif. Two disulfide bridges (Cys11 to Cys28 and Cys27 to Cys89 [33]) help to anchor the N-terminal region of the protein (residues 1 to 50) to the core of the enzyme.

The dinuclear copper site including a covalent cysteine-histidine bond

Both of the two copper atoms are coordinated by three histidine residues contributed from the four helices of the α-bundle (Figure 2 and 3). Both CuA and CuB are coordinated by N_ε of histidine residues, which are His88, His109, His118 to CuA, and His240, His244, His274 to CuB.

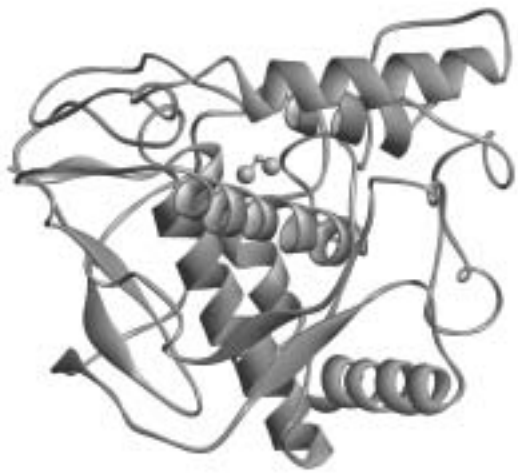

Figure 2: *Ribbon representation of ibCO.*

An interesting feature of the dinuclear metal center in ibCO is a covalent thioether bond formed between C_ε of CuA-coordinating His109, and S of Cys92. A cysteinyl-histidyl-bond has

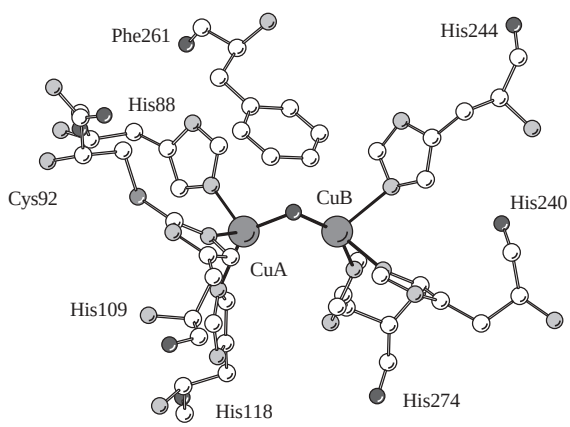

Figure 3: *Dinuclear metal site in ibCO.*

also been reported for TYR from *Neurospora crassa* [34] as well as for HC from *Helix pomatia* [35] and HC from *Octopus dofleini*. [31] However, the absence of the cysteinyl-histidyl bridge in HCs from arthropods and human TYR, does not support its direct involvement in the electron transfer process. The mononuclear copper enzyme galactose oxidase also exhibits a thioether bond between the C_ε carbon of a tyrosinate ligand and the sulphur of a cysteine to stabilize the tyrosine radical generated during catalysis. [36]

In the oxidized *met* CO structure the two cupric ions are 2.9 Å apart. [22,32] Most likely they are bridged by a hydroxide ion completing the four-coordinate coordination sphere. EPR data reflect an antiferromagnetically coupled EPR silent Cu(II)-Cu(II) state [12,27] of the enzyme which is in good agreement with a μ-hydroxo group bridging the copper atoms.

Crystal structure of the reduced form and the phenylthiourea inhibitor complexes

Reduced form: The crystal structure of the reduced form was solved with a resolution of 2.7 Å. Therefore, crystals of the native *met* form were soaked in 2 mM dithiothreitol (DTT) added to the mother liquor. Upon reduction of the enzyme to the Cu(I)-Cu(I) state the metal-metal separation extends significantly to 4.4 Å, whereas the histidines move very little. No significant conformational change is observed for the rest of the protein. The coordination spheres of the copper centers are changing to square planar with a vacant coordination site for CuB and to distorted trigonal pyramid with a coordinating water molecule for CuA. The coordinating residues are moving less indicating a rather rigid pocket.

Inhibitor complex: A crystal structure with a substrate analogue inhibitor was solved at a resolution of 2.7 Å. The inhibitor complex revealed conformational changes of the residues in the active site which indicated that access to the catalytic metal center is primarily controlled by rotation of the aromatic ring of Phe261 (see also Fig. 3). Upon binding of phenylthiourea (PTU) the phenyl ring of Phe261 and the imidazole ring of His244 undergo a conformational change to form hydrophobic interaction with the aromatic ring of the inhibitor. The sulphur of phenylthiourea replaces the hydroxo-bridge, present in the Cu(II)-Cu(II) enzyme, and coordinates both copper ions, thereby increasing the metal-metal separation to 4.2 Å. The amide nitrogen interacts weakly with CuB (Cu-N 2.6 Å) completing its square-pyramidal coordination sphere. In addition to the interaction with the dicopper center van der Waals interaction of the residues line the hydrophobic cavity (Phe261, Ile241, His244) contributing to the high affinity of PTU to the enzyme.

Reaction mechanism

A catalytic pathway for catechol oxidase was proposed on the basis of a combination of biochemical, spectroscopic and structural data (see Figure 4).

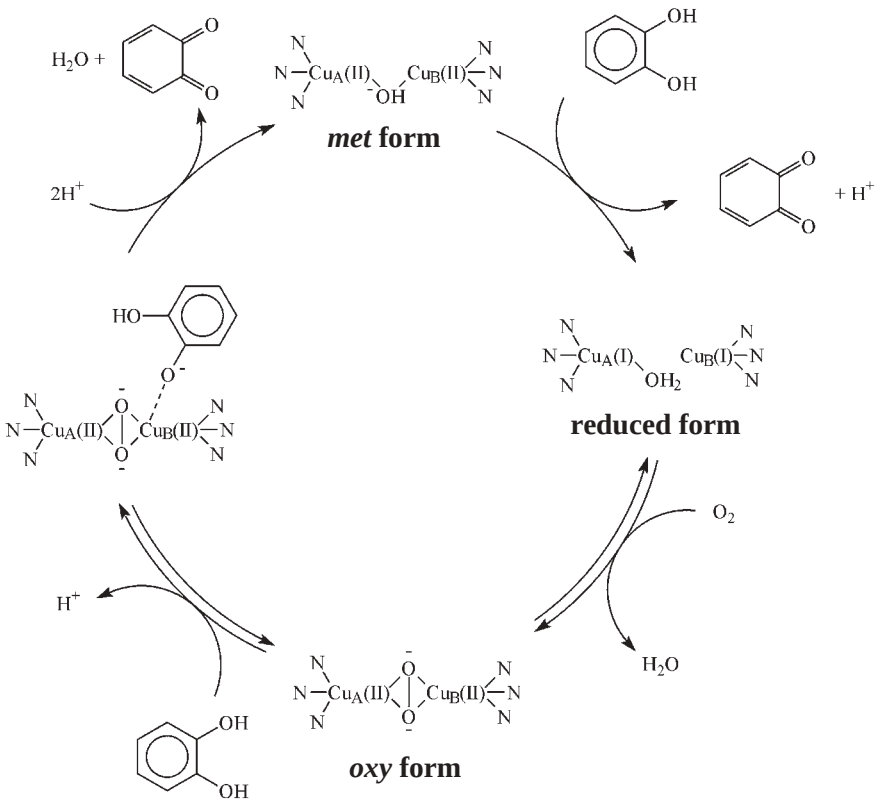

Figure 4: *Possible mechanism for ibCO, as proposed on the basis of structural, spectroscopic and biochemical data.*

In ibCO the rotation of the side chain of Phe261 that is positioned in the way the substrate must pass to reach the copper center opens the catalytic pocket of the copper center (not shown in Fig. 3) as it is positioned in the way the substrate must pass to reach the copper center. It therefore might influence substrate specificity of the enzyme by suppressing access of sterically pretentious substrates. Inside the pocket a glutamate residue Glu251 and an isoleucine residue Ile243 are positioned. The glutamate residue is supposed to be involved in dehydrogenating the substrate whereas the isoleucine residue seems to be involved in tuning the substrate specificity. Based on the binding mode observed for the phenylthiourea complex of CO a monodentate binding mode of the substrate is favored, in which the catechol substrate binds to CuB after deprotonation of one of the two hydroxyl groups. The first substrate molecule binds to the *met* form, which is the state present after purification. Once the quinone is formed the Cu center remains in its reduced state with one copper containing water molecule. Now oxygen binds to the reduced form. UV/Vis spectroscopic results in combination with Resonance Raman investigations suggests that molecular oxygen binds as peroxide in a μ-η^2:η^2 binding mode with a metal metal separation of 3.8 Å as determined by EXAFS spectroscopy. [27] The same binding mode for dioxygen has been determined for the oxygenated *Limulus polyphemus* HC with a Cu-Cu separation of 3.6 Å. [37] Binding of the second substrate molecule leads to a CO-O_2^{2-} substrate complex modeled with the aid of PTU*CO inhibitor complex.[32] In this model, CuB

would be six-coordinated with a tetragonal planar coordination by His240, His244 and the dioxygen molecule. The two axial positions would be occupied by His274 and the catechol substrate. The CuA site would have a tetragonal-pyramidal environment with His88, His118 and O_2^{2-} in equatorial positions, His109 taking the axial position, and a vacant non-solvent accessible sixth coordination site. In this proposed CO-O_2^{2-}-substrate complex, two electrons could be transferred from the second substrate molecule to the peroxide, followed by cleavage of the O-O bond, loss of water and departure of the o-quinone product.

The catalytic mechanism of TYR that also includes a mechanism for the catecholase activity of tyrosinase was studied by Solomon and coworkers. [38, 39, 40] This hypothesis expects the *oxy* form to be the starting point of cresolase activity. Monophenol substrate binds to the *oxy* form that is present in the resting form in a rate of about 15 % (85 % are in the met form) and is monooxygenated to the diphenol that subsequently binds in a bidentate fashion to the copper center in the *met* form. Oxidation of the diphenol substrate leads to the reduced form of the dinuclear copper center. Transformation from the reduced form to the *oxy* form happens due to attack of dioxygen and closes the catalytic cycle.

Catecholase activity would therefore be possible to start from both oxy and met form. Diphenol substrate binds to the met form (for example) followed by oxidation to the first quinone and the reduced form. Analogous to the above mentioned, dioxygen forms the *oxy* form again that is attacked by the second diphenol molecule. Oxidation to the quinone leads to the met form again and closes the catalytic cycle. The mechanism by Solomon is very similar to the one of CO, described above.

Additionally, a radical mechanism has been favoured by Kitajima and coworkers [41, 42] and a Cu(III) intermediate based on model compound investigations was suggested by Holland [43, 44] and Itoh. [45] These suggestions might also be important for the mechanism of CO, but with the absence of a crystal structure of TYR it is difficult to enlighten distinct mechanistic differences of CO and TYR.

Physiological Role

There were several functions proposed for CO in higher plants, but none of them is really convincing. A long discussed possible function of plant CO is a role in disease defense of higher plants. [46] The enzyme is cytosolic or membrane-bound whereas possible substrates are kept separated in the vacuole. After disruption of the cell by wounding or infection the membrane is lysed and these two components get in contact forming quinones to polymerize to melanins. CO mRNA has been found to be upregulated after wounding or infecting in apples. [47] Furthermore, some parasites have been found to use inhibitors of the CO indicating the CO/diphenol system to be a hurdle to take for colonization of the parasite's host.

Other proposed physiological roles are pigment formation and oxygen scavenging in the chloroplast. [46, 48, 49] Plant CO have often been found to be tightly bound to the thylakoid membranes and also to have transit peptides leading to the plastide membrane. There is some evidence for a role in photosynthesis as CO has been found to be nuclear encoded but still inactive until incorporated into the plastids. There it is supposed to be involved to the Mehler reaction.

In mammals TYR starts forming skin pigmentation. [46] The absence or inactivation of the enzyme therefore leads to forms of albinism (tyrosinase-negative albinism and ocultaneous albinism). In insects TYRs are involved both in sclerotization and defense.

Bioinorganic approaches for type 3 copper proteins
Dinuclear peroxo copper(II) compounds

A wide range of work has been done in the field of biomimetic model compounds for type 3 copper proteins that has been summarized in a number of articles. [26, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60] Model compounds of the active site of copper type-3 proteins played an important role in elucidating the binding mode of dioxygen in the oxy form.

In 1988 Karlin reported the first crystal structure for a peroxo copper(II) complex using the ligand tris[(2-pyridyl)methyl]amine (tpa). In a self assembly reaction of the monomeric Cu(I)(tpa) precursors with molecular oxygen the thermally unstable complex $[(Cu(tpa))_2(O_2)]^{2+}$ was obtained at low temperatures (see Figure 5.).

The peroxo group is bound in a *trans* μ-1,2 bridging mode. The Cu(II)-Cu(II) distance in this compound is 4.36 Å with an O-O bond length of 1.43 Å. The copper(II) ions are surrounded in a trigonal bipyramidal fashion with one peroxide oxygen and the amine nitrogen taking the axial positions. The unpaired electrons are located in the d_z^2 orbitals in the direction of the Cu-O bond, and are strongly antiferromagnetically coupled. [61]

The UV/Vis-spectrum of the intensively purple colored compound shows absorption bands at 440 nm ($\varepsilon = 2000\ mol^{-1}cm^{-1}$), 525 nm ($\varepsilon = 11500\ mol^{-1}cm^{-1}$) and 590 nm ($\varepsilon = 7600\ mol^{-1}cm^{-1}$), that can be attributed to $O_2^{2-} \rightarrow$ Cu(II) charge transfer transitions. Another band at 1035 nm ($\varepsilon = 180\ mol^{-1}\ cm^{-1}$) is assigned to a d-d transition.

Figure 5: *structure of the complex cation* $[(Cu(tpa))_2(O_2)]^{2+}$*; hydrogen atoms omitted for clarity [61]*

The O-O stretching vibration frequency was determined to be 832 cm^{-1} by Resonance Raman studies. [62] This complex represents the first functional model for oxy hemocyanin, the spectroscopic and structural qualities, however, do not fit to those of the protein.

In 1989 Kitajima [26, 63] succeeded with the first synthesis of a side on μ-η^2:η^2 peroxo copper(II) complex employing the ligand hydrotris(3,5-diisopropyl-1-pyrazolyl)borate (HB(3,5-iPr2pz)3) (see Figure 6).

Similar complexes had only been described for f-block elements at that time. [64, 65] Preparation of the complex was performed at low temperatures either by the reaction of a monomeric Cu(I) complex with O_2 or by adding excess of H_2O_2 to dinuclear µ-oxo or di-µ-hydroxo bridged copper(II) complexes. The copper ions are located in a distorted square pyramidal environment with an intermetallic distance of 3.56 Å. The O-O bond length in this compound is 1.41 Å. UV/Vis spectroscopic investigations of the intensively violet colored compound revealed $O_2^{2-} \rightarrow$ Cu (II) charge transfer bands at 349 nm (ε = 21000 mol^{-1} cm^{-1}) and 551 nm (ε = 790 mol^{-1} cm^{-1}) and the frequency of the O-O streching vibration was determined in Resonance Raman experiments to be 741 cm^{-1}. Magnetic susceptibility measurements suggest a strong antiferromagnetic coupling of the copper(II) ions. [66] This model compound exhibits similar magnetic, spectroscopic and structural features compared to the *oxy* form of HC. Thus it contributes significantly to the elucidation of the structural and functional properties of the oxygen binding in type 3 copper proteins.

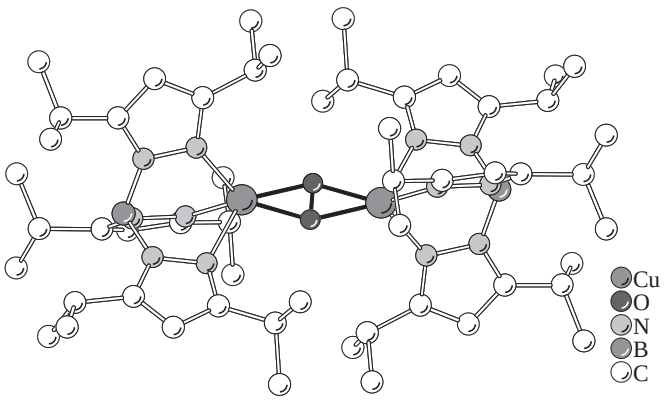

Figure 6: *structure of the complex* $[(Cu(HB(3,5\text{-}iPr_2pz)_3))_2(O_2)]^{2+}$*; hydrogen atoms omitted for clarity [26, 63]*

Gorun et al. were able to obtain the crystal structure of another side on μ-η^2:η^2 peroxo copper(II) complex with the modified ligand hydrotris(3-trisfluoro-5-methyl-1-pyrazolyl)borate in 2001.[67]

The first room temperature stable side on μ-η^2:η^2 peroxo copper(II) complex was reported by Suzuki et al. in 1999. It contained a dinucleating ligand that provides three aromatic nitrogen donor atoms for each copper atom.[68]

Tetranuclear *peroxo* copper(II) compounds

Krebs et al. presented a new type of peroxide binding in copper(II)-complexes of the ligand 4-methyl-2,6-bis(pyrrolidinomethyl)-phenolate (mbpmp) in 1994. The tetranuclear compound contained a fourfold bridging end on peroxo group in a μ_4-(η^1) coordination mode (see Figure 7) [69].

At that time this compound was the only known room temperature stable peroxo copper (II) complex. The μ_4-coordination had only been described for a tetranuclear molybdenum complex and a hexanuclear iron complex, previously.[70, 71]

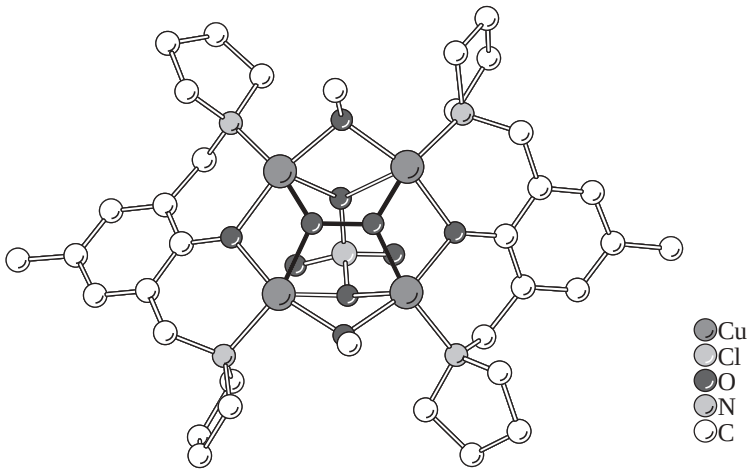

Figure 7: *structure of the complex cation $[(Cu_2(mbpmp))_2(O_2)(OMe)_2(ClO_4)]^+$; hydrogen atoms omitted for clarity [69]*

Meyer et al. recently synthesized another complex with this unusual μ_4-(η^1) peroxo coordination mode. [72] (see Figure 8).

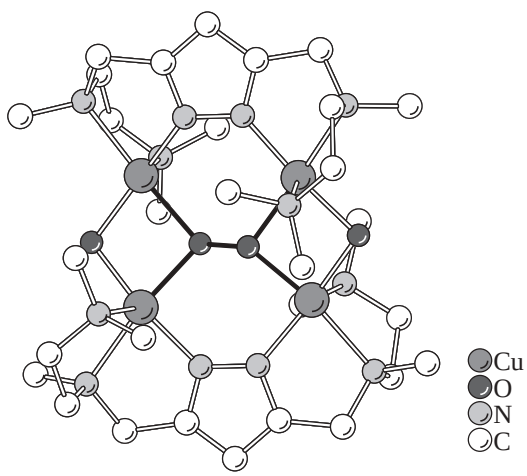

Figure 8: *structure of the complex cation $[(Cu_2(L_{pz}))_2(O_2)(OH)_2]^{2+}$; hydrogen atoms omitted for clarity [72]*

This copper compound is based on the pyrazol ligand 3,5-bis(1-methyl-4-dimethyl-diaminoethyl)pyrazol (L_{pz}) and its geometry differs from that of the tetranuclear core reported by Krebs. In that compound the peroxo ligand caps a nearly planar Cu_4 rectangle and thus adopts a *cis* configuration with respect to the O-O bond. In Meyer's complex the oxygen atoms of the

O_2^{2-} group are situated on different sides of the Cu_4 framework, yielding a *trans* arrangement of the metal centers.

Dinuclear chloro copper complexes

The synthesis of dinuclear copper compounds with coordinated chlorides is interesting for different reasons. On one hand these complexes are biomimetic models for the binding of chloride to tyrosinase or catechol oxidase, that was reported by Canters et al., [19] on the other hand such compounds allow to predict whether the utilized ligands offer the possibility to synthesize peroxo complexes or not.

Roundhill et al. presented a bis(μ_2-chloro)complex based on the ligand hydro-tris(1-pyrazolyl)borate ($HB(1\text{-}pz)_3$) in 1979 (see Figure 9) [73]. Reaction of copper(II) chloride with $Na(HB(1\text{-}pz)_3)$ yielded the symmetric dinuclear compound $[(Cu(HB(1\text{-}pz)_3))_2(\mu\text{-}Cl)_2]$.

Also triazacyclic ligands have been used in the past to synthesize dinuclear μ-chloro copper complexes. [74, 75, 76, 77] The first crystal structure of a copper compound exhibiting this motive was presented by Weatherburn in 1990. [78] Each of the two copper centers is coordinated by three nitrogen atoms of the ligand 1,5,9-triazacyclododecan and two bridging ligands, a hydroxide and a chloride.

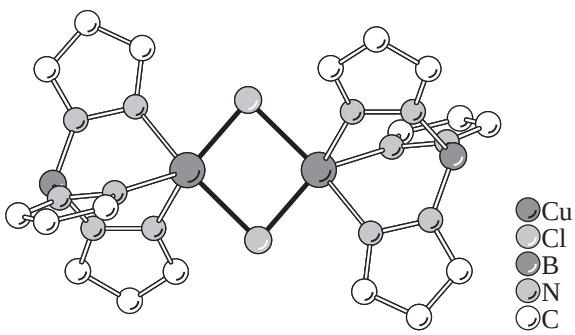

Figure 9: *structure of the complex $[(Cu(HB(1\text{-}pz)_3))_2(\mu\text{-}Cl)_2]$; hydrogen atoms omitted for clarity; only one position of the disordered peroxo group shown [73]*

Cresolase activity of copper complexes

With the complex $[Cu_2(L\text{-}66)]^{2+}$ (with L-66 = α,α'-bis[bis[2-(1'-methyl-2'-benzimidazolyl)ethyl]amino]-m-xylene Casella et al. presented the first model compound that was able to bind dioxygen reversibly followed by oxidation of a phenol to the corresponding catechol. [79] Temperature variations between –80 and –45 °C caused reversible uptake of dioxygen to form a peroxo copper(II) species, repeatedly. At –60 °C the compound was able to oxidize the added substrate 4-methoxyphenol to the corresponding catechol. This biomimetic model complex represents the first operating tyrosinase model system, that imitates the cresolase activity of metallo enzymes.

Some working groups deal with the synthesis of model complexes for the binding of the phenolic oxygen in the course of the catalytic cycle of tyrosinase. Neves et al. synthesized a complex based on the asymmetric ligand N-(2-hydroxybenzyl)-N,N',N'-tris[(2-pyridyl)methyl]-1,3-diaminopropan-2-ol (Hbtppnol) containing a non deprotonated phenol group that occupies

an apical position on one of the two copper atoms (see Figure 10). [80, 81, 82] This complex can be regarded as a structural model compound for an proposed intermediate during the hydroxylation of monophenols to catechols by tyrosinase.

Catecholase activity of copper compounds

Systematic investigations towards the catecholase activity of copper complexes have been carried out for the first time by Nishida in 1980 [83]. Mononuclear square planar copper(II) complexes show only a low catalytic activity, whereas non planar mononuclear complexes exhibit a high activity. If the copper copper distance is smaller than 5 Å, high activities can also be achieved with dinuclear compounds. This is explained by the necessity of a steric match between the substrate and the complex, like a Cu-Cu distance that fits to the bite of the catecholate

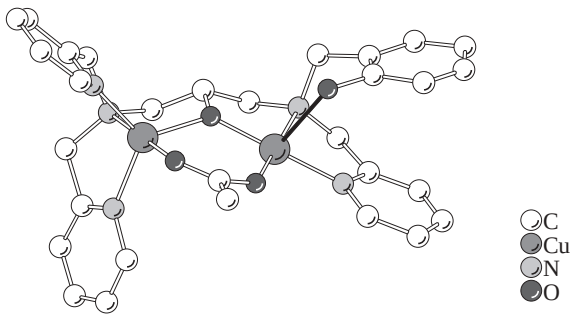

Figure 10: *structure of the complex cation $[Cu_2(Hbtppnol)(CH_3COO)]^{2+}$; hydrogen atoms omitted for clarity [81, 82]*

Other working groups supported this thesis by showing that dinuclear copper complexes are in general more active than comparable mononuclear ones. [84, 85]

So far, only a few crystal structures of catalytically active dinuclear complexes have been described, that exhibit a coordinated catechol or quinone ligand. In Karlin's complex $[Cu_2(L\text{-}O)(tcc)]^+$ (where $L\text{-}O^-$ = 2,6-bis[[bis((2-pyridyl)methyl)amino]methyl]phenolate and tcc = tetrachlorocatecholate dianion)

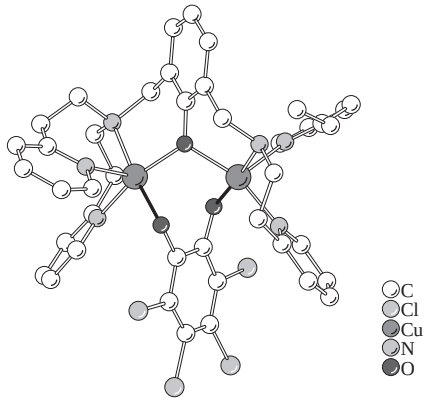

Figure 11: *structure of the complex cation $[Cu_2(L\text{-}O^-)(tcc)]$; hydrogen atoms omitted for clarity [83]*

the two oxygen atoms of the tetrachlorocatecholate ligand are coordinated to different copper ions leading to a square-pyramidal coordination sphere at each metal center (see Figure 11). [86]

In 2002 Meyer et al. found a different coordination mode for the catecholate in copper complexes utilizing dinucleating pyrazol-based ligands (see Figure 12). [87]

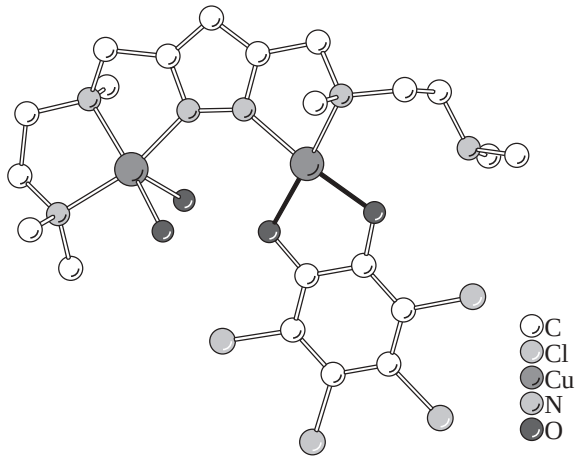

Figure 12: *structure of the complex cation* $[(Cu_2(L_{pz})(tcc)(H_2O)_2]^{2+}$*; hydrogen atoms omitted for clarity [87]*

The catecholate is bound bidentately to only one copper ion in these model complexes.

Acknowledgements

Annette Rompel thanks the CNRS and the DFG for financial support of the project: Learning from Nature how two metal centers operate: Studies of purple acid phosphatase (PAP) and polyphenol oxidases (PPO) - enzymes and models.

References

[1] Mason, H.S. *Nature* **1956**, *177*, 79.
[2] Bonaventura, J.; Bonaventura, C. *Am. Zool.* **1980**, *20*, 7.
[3] Herskovitz, T.T. *Comp. Biochem. Physiol*. **1988**, *91*, 597.
[4] Kubowitz, F. *Biochem. Z.*, **1937**, *292*, 222.
[5] Kubowitz, F. *Biochem. Z.*, **1938**, *299*, 32.
[6] Mayer, A.M. Harel, E. *Phytochemistry*, **1979**, *18*, 193.
[7] Jiang, Y.M.; Fu, J.R.; Zauberman, G.; Fuchs, Y. *J. Sci. Food Agricult.*, **1999**, *79*, 950
[8] Paul, B.; Gowda, L. R. *J. Agric. Food Chem.*, **2000**, *48*, 3839.
[9] Yang, C.P.; Fujita, S.; Ashrafuzzaman, M.; Nakamura, N.; Hayashi, N. *J. Agric. Food Chem.*, **2000**, *48*, 2732.
[10] Bilka, F.; Balazova, A.; Bilkova, A.; Psenak, M. *Pharmazie*, **2000**, *55*, 155.
[11] Mazzafera, P.; Robinson, S.P. *Phytochemistry*, **2000**, *55*, 285.
[12] Rompel, A.; Fischer, H.; Meiwes, D.; Büldt-Karentzopoulos, K.; Dillinger, R.; Tuczek, F.; Witzel, H.; Krebs, B. *J. Biol. Inorg. Chem.*, **1999**, *4*, 56.

[13] Sheptovitsky, Y.G.; Brudvig, G.W. *Biochemistry*, **1996**, *35*, 16255.
[14] Royo, J.B.; Cabello, F.; Miranda, S.; Gogorcena, Y.; Gonzalez, J.; Moreno, S.; Itoiz, R. Ortiz, J.M. *Sci. Hortic.*, **1997**, *69*, 145.
[15] Jolley Jr, R.J.; Evans, L.H.; Mason, H.S. *Biochem. Biophys. Res. Commun*. **1972**, *46*, 878.
[16] Jolley Jr, R.J.; Evans, L.H.; Makino, N.; Mason, H.S. *J. Biol. Chem.* **1974**, *249*, 335.
[17] Himmelwright, R.S.; Eickman, N.C.; LuBien, C.D.; Lerch, K.; Solomon, E.I. *J. Am. Chem. Soc.* **1980,** *102*, 7339.
[18] Eickman, N.C.; Himmelwright, R.S.; Solomon, E.I. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 2094.
[19] Bubacco, L.; Salgado, J.; Tepper, A.W.J.W.; Vijgenboom, E.; Canters, G.W. *FEBS Lett.* **1999**, *442*, 215.
[20] Woolery, G.L.; Powers, L.; Winkler, M.; Solomon, E.I.; Spiro, T.G. *J. Am. Chem. Soc.* **1984**, *106*, 86.
[21] Co, M.S.; Hodgson, K.O.; Eccles, T.K.; Lontie, R. *J. Am. Chem. Soc.* **1981,** *103*, 984.
[22] Co, M.S.; Hodgson, K.O. *J. Am. Chem. Soc.* **1981,** *103*, 3200.
[23] Rompel, A.; Fischer, H.; Büldt-Karentzopoulos, K.; Meiwes, D.; Zippel, F.; Nolting, H.-F.; Hermes, C.; Krebs, B.; Witzel, H. *J. Inorg. Biochem.* **1995**, *59*, 715.
[24] Eickman, N.C.; Solomon, E.I.; Larrabee, J.A.; Spiro, T.G.; Lerch, K. *J. Am. Chem. Soc.* **1978**, *100*, 6529.
[25] Ling, J.; Nestor, L.P.; Czernuszewicz, R.S.; Spiro, T.G.; Fraczkiewicz, R.; Sharma, K.D.; Loehr, T.M.; Sanders-Loehr, J. *J. Am. Chem. Soc.* **1994**, *116*, 7682.
[26] Kitajima, N.; Fujisawa, K.; Moro-oka, Y. *J. Am. Chem. Soc.* **1989**, *111*, 8975.
[27] Eicken, C.; Zippel, F.; Büldt-Karentzopoulos, K.; Krebs, B. *FEBS Lett.* **1998**, *436*, 293.
[28] Himmelwright, R.S.; Eickman, N.C.; LuBien, C.D.; Solomon, E. I. *J. Am. Chem. Soc.*, **1980**, *102*, 5378.
[29] Gaykema, W.P.; Volbeda, A.; Hol, W.G. *J. Mol. Biol.* **1986**, *187*, 255.
[30] Hazes, B.; Magnus, K.A.; Bonaventura, C.; Bonaventura, J.; Dauter, Z.; Kalk, K.H.; Hol, W.G. *Protein Sci.* **1993**, *2*, 597.
[31] Cuff, M.E.; Miller, K.I.; van Holde, K.E.; Hendrickson, W.A. *J. Mol. Biol.* **1998**, *278*, 855.
[32] Klabunde, T.; Eicken, C.; Sacchettini, J.C.; Krebs, B. *Nat. Struct. Biol.* **1998**, *5*, 1084.
[33] Gerdemann, C.; Eicken, C.; Meyer, H.; Spener, F.; Krebs, B. EMBL database, **1998** accession number AJ006097.
[34] Lerch, K. *J. Biol. Chem.* **1982**, *257*, 6414.
[35] Gielens, C.; De Geest, N.; Xin, X.Q.; Devreese, B.; Van Beeumen, J.; Preaux. G. *Eur. J. Biochem.* **1997**, *248*, 879.
[36] Ito, N.; Phillips, S.E.V.; Stevens, C.; Ogel, Z.B.; McPherson, M.J.; Keen, J.N.; Yadav, K.D.S.; Knowles, P.F. *Nature* **1991**, *350*, 87.
[37] Magnus, K.; Ton-That, H. *J. Inorg. Biochem.* **1992**, *47*, 20.
[38] Wilcox, D.E.; Porras, A.G.; Hwang, Y.-T.; Lerch, K.; Winkler, M.E.; Solomon, E.I. *J. Am. Chem. Soc.* **1985**, *107*, 4015.
[39] Solomon, E.I.; Lowery, M.D. *Science* **1993**, *259*, 1575.

[40] Solomon, E.I.; Sundaram, U.M.; Machonkin, T.E. *Chem. Rev.* **1996**, *96*, 2563.
[41] Kitajima, N. 1993. In: K. D. Karlin, Z. Tyéklar, eds. *Bioinorganic Chemistry of Copper*. Chapman and Hall, New York. 251-263.
[42] Moro-oka, Y.; Fujisawa, K.; Kitajima, N. *Pure Appl. Chem.* **1995**, *67*, 241.
[43] Holland, P.L.; Rodgers, K.R.; Tolman, W.B. *Angew. Chem. Int. Ed.* **1999,** *38*, 1139.
[44] Holland, P.L.; Tolman, W.B. *Coord. Chem. Rev.* **1999**, *192*, 855.
[45] Itoh, S.; Taki, M.; Nakao, H.; Holland, P.L.; Tolman, W.B.; Que Jr., L.; Fukuzumi, S. *Angew. Chem. Int. Ed.* **2000**, *39*, 398.
[46] Walker, J.R.; Ferrar, P.H.; *Biotechnol. Genet. Eng. Rev.* **1998**, *15*, 457.
[47] Boss, P.K.; Gaudner, R.C.; Janssen, B.-J.; Ross; G. S. *Plant Mol. Biol*. **1995**, *27*, 429
[48] Vaugh, K.C.; Duke, S.O. *Physiol. Plant* **1984**, *60*, 106.
[49] Vaugh, K.C.; Lax, A.R.; Duke, S.O. *Physiol. Plant* **1988**, *72*, 659.
[50] Karlin, K.D.; Kaderli, S.; Zuberbühler, A.D. *Acc. Chem. Res.* **1997**, *30*, 139.
[51] Fox, S.; Karlin, K. D. *Active Oxygen in Biochemistry* (Eds. J. S. Valentine, C. S. Foote, A. Greenberg, J. F. Liebman), Chapman & Hall: Glasgow, Scotland **1995**, 188.
[52] Kitajima, N.; Tolman, W.B. *Prog. Inorg. Chem.* **1995**, *43*, 419.
[53] Tolman, W.B. *Acc. Chem. Res.* **1997**, *30*, 227.
[54] Solomon, E.I.; Tuczek, F.; Root, D.E.; Brown, C.A. *Chem. Rev.* **1994**, *94*, 827.
[55] Kitajima, N.; Moro-oka, Y. *Chem. Rev.* **1994**, *94*, 737.
[56] Karlin, K.D.; Tyeklár, Z. *Adv. Inorg. Biochem* **1994**, *9*, 123.
[57] Kitajima, N.; Moro-oka, Y. *J. Chem. Soc., Dalton Trans.* **1993**, 2665.
[58] Sorrell, T.N. *Tetrahedron* **1989**, *45*, 3.
[59] Tyeklár, Z.; Karlin, K.D. *Acc. Chem. Res.* **1989**, *22*, 241.
[60] Karlin, K.D.; Gultneh, Y. *Prog. Inorg. Chem.* **1987**, *35*, 219.
[61] Karlin, K.D.; Tyeklár, Z.; Farooq, A.; Jacobson, R.R.; Sinn, E.; Lee, D.W.J.; Bradshaw, E.; Wilson, L.J. *Inorg. Chim. Acta* **1991**, *182*, 1.
[62] Baldwin, M.J.; Ross, P.K.; Pate, J.E.; Tyeklár, Z.; Karlin, K.D.; Solomon, E.I.; *J. Am. Chem. Soc.* **1991**, *113*, 8671.
[63] Kitajima, N.; Fujisawa, K.; Fujimoto, C.; Moro-oka, Y.; Hashimoto, S.; Kitagawa, T. Toriumi, K.; Tatsumi, K.; Nakamura, A. *J. Am. Chem. Soc.* **1992**, *114*, 1277.
[64] Lapshin, A.E.; Smolin, Y.J.; Shepelev, Y.F. *Acta Chryst.* **1990**, *C46*, 1753.
Bradley, D.C.; Ghotra, J.S.; Hart, F.A.; Hursthouse, M.B.; Raithby, P.R. *J. Chem. Soc., Dalton Trans.* **1977**, 1166.
[65] Haegele, R.; Boeyens, J.C. *J. Chem. Soc., Dalton Trans.* **1977**, 648.
[66] Baldwin, M.J.; Root, D.E.; Pate, J.E.; Fujisawa, K.; Kitajima, N.; Solomon, E.I. *J. Am. Chem. Soc.* **1992**, *114*, 10421.
[67] Hu, Z.; George, G.N.; Gorun, S.M. *Inorg. Chem.* **2001**, *40*, 4812.
[68] Kodera, M.; Katayama, K.; Tachi, Y.; Kano, K.; Hirota, S.; Fujinami, S.; Suzuki, M. *J. Am. Chem. Soc.* **1999**, *121*, 11006.
[69] Reim, J.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1969.
[70] Stomberg, R.; Trysberg, L.; Larking, I. Acta Chem. Scand. **1970**, *24*, 2678.
[71] Micklitz, W.; Bott, S.G.; Bentsen, J.G.; Lippard, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 372.

[72] Meyer, F.; Pritzkow, H. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 2112.
[73] Roundhill, S.G.N.; Roundhill, D.M.; Bloomquist, D.R.; Landee, C.; Willett, R.D.; Dooley, D. M.; Gray, H.B. *Inorg. Chem.* **1979**, *18*, 831.
[74] Elliot, D.J.; Martin, L.L.; *Z. Kristallogr. – New Crystal Structures* **1999**, *214*, 38.
[75] Burger, K.-S.; Chaudhuri, P.; Wieghardt, K. *Inorg. Chem.* **1996**, *35*, 2704.
[76] Kohn, R.D.; Seifert, G.; Kociok-Kohn, G. *Chem. Ber.* **1996**, *129*, 1327.
[77] Chen, X.-M.; Yao, Y.-X.; Xu, Z.-T.; Shi, K.-L.; Xu, Y.-J. *Polyhedron* **1995**, *14*, 1195.
[78] Graham, P.G.; Weatherburn, D.C.; March, F.C.; Robinson, W.T. *Inorg. Chim. Acta* **1990**, *178*, 227.
[79] Santagostini, L.; Gullotti, M.; Monzani, E.; Casella, L.; Dillinger, R.; Tuczek, F. *Chem. Eur. J.* **2000**, *6*, 519.
[80] Gentschev, P.; Lüken, M.; Möller, N.; Rompel, A.; Krebs, B. *Inorg. Chem. Commun.* **2001**, *4*, 753.
[81] Neves, A.; Rossi, L.M.; Horn Jr., A.; Vencato, I.; Bortoluzzi, A.J.; Zucco, C.; A. S. Mangrich, *Inorg. Chem. Comm.* **1999**, *2*, 334.
[82] Neves, A.; Rossi, L.M.; Vencato, I.; Drago, V.; Haase, W.; Werner, R. *Inorg. Chim. Acta* **1998**, *281*, 111.
[83] Oishi, N.; Nishida, Y.; Ida, K.; Kida, S. *Bull. Chem. Soc. Jpn.*, **1980**, *53*, 2847.
[84] Casellato, U.; Tamburini, S.; Vigato, P.A.; de Stefani, A.; Vidali, M.; Fenton, D.E. *Inorg. Chim. Acta* **1983**, *69*, 45.
[85] Malachowski, M.R.; Davidson, M.G. *Inorg. Chim. Acta* **1989**, *162*, 199.
[86] Karlin, K.D.; Gultneh, Y.; Nicholson, T.; Zubieta, J. *Inorg. Chem.* **1985**, *24*, 3727.
[87] Ackermann, J.; Meyer, F.; Kiefer, E.; Pritzkow, H. *Chem. Eur. J.* **2002**, *8*, 247.