1. Introduction

Copper plays an important role in various biological processes occurring in higher plants and animals. In its cupric form it is an important constituent of active sites in metalloenzymes and proteins. Most of these copper-containing enzymes are involved in redox processes like electron-transfer, dioxygen chemistry, reduction of nitrogen oxides, including nitrite and nitrous oxide. By performing this kind of redox processes, copper proteins take over similar functions as iron containing metalloenzymes, i.e. hemocyanines are blood O_2-carriers in arthropods and mollusks, and hemoglobin, an iron containing heme-enzyme, is the blood O_2-carrier in the human body. However, copper and iron enzymes feature some differences such as the molecular structure of the active sites or the redox potentials.[1]

For humans copper is an essential element, too, and with approximately 3 mg per kg body weight the third most abundant trace element in the human body.[2] Serious diseases can occur when the human copper metabolism is perturbated. Wilson's disease describes the incapability of copper release.[3] Without medical treatment Wilson's disease leads to an accumulation of copper in the liver and the brain which finally leads to death. Menke's syndrome describes the insufficient copper storage in cells.[4-6] This hereditary syndrome leads to anemia and the degeneration of the nervous system which can be lethal if the syndrome stays untreated.

The most common oxidation states of copper ions which are involved in biological processes are +1 and +2. According to the PEARSON concept copper(I) ions show soft lewis acid behavior whereas copper(II) ions can be classified as relatively hard acids. This results in different preferences of the coordinated donor atom in coordination compounds. The oxidation state and the resulting electronic configuration of the ion also effects the coordination geometries.[7]

The electronic configuration of copper(I) ions is d^{10}. In this closed shell system all electrons are paired. The preferred coordination numbers of copper(I) ions and the corresponding geometries are 2 (linear), 3 (trigonal-planar) and 4 (tetrahedral).[7] The soft copper(I) ions prefer sulfur ligands and softer N-donor ligands such as pyridines, imidazoles or nitriles in coordination compounds.

Cupric ions feature an electronic d^9-configuration. The preferred coordination numbers and polyhedra are 4 in a square planar arrangement, 5 in either square pyramidal or trigonal bipyramidal configuration or 6 in a Jahn-Teller distorted octahedral coordination sphere.[8] Copper(II) ions prefer aliphatic amines and anions as donor sets, i. e. carboxylate or deprotonated amidine units, in coordination compounds.

According to the electronic properties of the copper ions, the resulting complexes show different spectroscopic features. Copper(I) ions are spectroscopically silent because of their closed shell d^{10}-configuration, whereas copper(II) ions possess one single electron and show interesting spectroscopic and magnetic features.

The behavior of metal ions in proteins cannot be completely separated from the fundamental chemistry of the particular metal. Therefore the synthesis and characterization of bioinspired copper complexes represents an interesting field in synthetic copper coordination chemistry.

2. Current state of research

2.1. Metalloenzymes with a copper containing active site

In nature metalloenzymes with a copper containing active site play a crucial role in metabolic and cellular processes of eucaryotes and procaryotes. They take over a wide range of functions, such as electron transfer, oxygen transport, the oxidation of organic substrates or the reduction of small inorganic molecules.[2]

Historically, copper proteins have been divided into three different subclasses, according to their structural and spectroscopic features. The first class are the so called *type I* copper proteins. These proteins employ one copper ion in their active site which is coordinated by two histidine residues and one cysteinato residue. The strongly distorted tetrahedral coordination sphere is usually completed by a weakly bound methionine residue (Figure 2.1). *Type I* proteins are also called blue copper proteins due to their characteristic blue color in the cupric state. This color originates from a LMCT (ligand to metal charge transfer) of the cysteinato-residue to the copper(II) ion. In nature, *type I* copper proteins are responsible for electron transfer, e.g. in herbal or bacterial photosynthesis.[9]

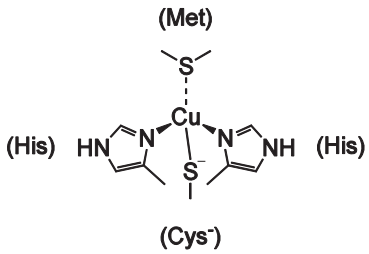


Figure 2.1: Structural constitution of *type I* copper proteins.

Type II copper active sites describe mononuclear copper proteins with an N or N/O ligand sphere (Figure 2.2). They exhibit an EPR-signal that is typical for copper(II) ions in coordination compounds. A weak d-d electronic transition of the cupric ion leads to the characteristic light blue color of these enzymes in the oxidized state. The enzyme class plays an important role in the activation of dioxygen in the presence of organic coenzymes.[9]

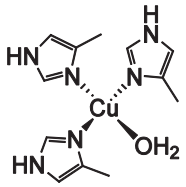
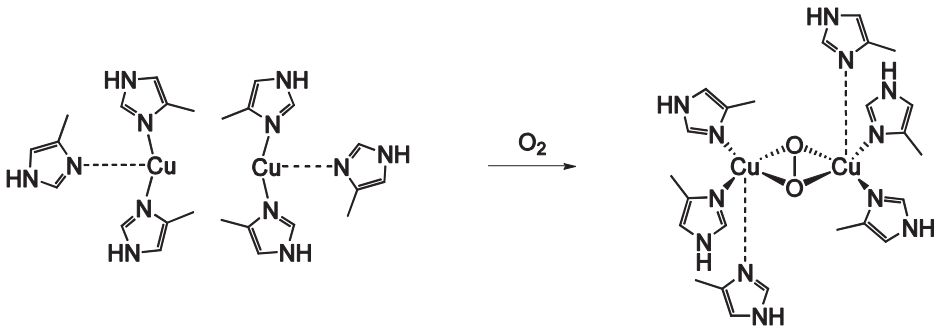


Figure 2.2: Structural constitution of *type II* copper proteins.

Type III copper enzymes exhibit a binuclear copper core. Each copper atom is coordinated by two strongly bound and one weakly coordinated histidine residue (Scheme 2.1). The vacant coordination site of the copper ions is utilized to incorporate oxygen and substrates between the metal centers, which is accompanied by an oxidation of the copper ions. *Type III* copper proteins are either involved in oxygen transport (e.g. hemocyanine) or in the oxidation and oxygenation of organic substrates (e.g. catechol oxidase or tyrosinase).[2, 9-10]



Scheme 2.1: Structural constitution of *type III* copper proteins (hemocyanine as the example). Left: desoxy form, right: oxy form.[2]

In the last decades further copper containing enzymes have been identified, for example the mixed-valent Cu_A-site, the Cu_B-$heme_A$ center of cytochrome *c* oxidase, the Cu_Z-center of nitrous oxide reductase and the multinuclear centers of metallothioneines, to mention the most important.

The Cu_A-center is a binuclear mixed-valent copper-cluster that is bridged by two cysteinato residues. The Cu_A-cluster was first identified in cytochrome *c* oxidase. Cu_A is responsible for electron delivery in the catalytic process of the reduction of molecular dioxygen to water.[10]

The second site of cytochrome *c* oxidase, the Cu_B-center, exhibits a single copper ion, which is coordinated by either two or three histidine residues. The Cu_B-center interacts

with a heme$_A$-iron-center in the catalytic process. Cytochrome *c* oxidases are involved in the eucariotic respiratory process.[10]

Metallothioneines (MT) are low molecular weight proteins that are characterized by their high percentage of cysteine; they bind up to eight cysteinato-bridged copper ions. MTs are responsible for copper transport and copper storage in humans and animals. Additionally it is assumed that they have a detoxifying protection function towards divalent metal ions like cadmium, copper and mercury.[2, 11]

Recently a further novel copper containing enzyme was described. Nitrous oxide reductase (N_2OR) is involved in the nitrogen cycle, it decomposes nitrous oxide (N_2O). The enzyme employs two different copper-containing active sites, the already mentioned Cu_A-center[12] and the Cu_Z-center. The Cu_Z-center contains a unique structure with four copper ions and two inorganic sulfido ligands.[13]

Due to the exceptional structure of the Cu_Z-site and the biological role of the enzyme N_2OR stands in the spotlight of modern biological and bioinorganic research.

2.2. The N_2O-reductase (N_2OR)

Nitrous oxide (N_2O) is an important component of earth's atmosphere. It is part of the natural denitrification cycle[14] and one of the most important greenhouse gases.[15] Although its percentage is much lower than that of methane or carbon dioxide, the warming potential of N_2O is 310 times higher than the one of CO_2.[15] Furthermore it has been identified as one of the most important ozone depleting substances recently.[16-17] Although the decomposition of N_2O to yield dinitrogen and dioxygen (Scheme 2.2) is thermodynamically favorable, the molecule is kinetically inert due to the high activation barrier of 250 kJ·mol^{-1} because the process is spin-forbidden. In nature the enzyme N_2OR performs the two electron reduction of N_2O to yield dinitrogen and water (Scheme 2.2).

$$N_2O \rightarrow N_2 + ½\ O_2$$

$$N_2O + 2\ H^+ + 2\ e^- \rightarrow N_2 + H_2O$$

Scheme 2.2: Decomposition pathways of N_2O, top: thermal decomposition, bottom: catalyzed decomposition.

N_2OR was isolated in 1982[18] for the first time and it was proposed that the enzyme contains two distinct copper sites, the Cu_A-center and the Cu_Z-center. The Cu_A-center delivers the electrons for the reduction process and the reaction proceeds at the Cu_Z-center.

In 1991 FARRAR *et al*. suggested two different coexisting forms of the Cu_Z-site in the enzyme, namely the redox-active form Cu_Z and the inactive form Cu_Z^*.[19] The corresponding percentage of each form depends on the purification method.[20] In case of an anaerobic purification method the Cu_Z-form is enriched while aerobic purification methods lead to a higher percentage of the Cu_Z^*-form. The two forms are often distinguished according to their spectroscopic features into the purple, anaerobic form and the pink, aerobic form.[21]

The first N_2OR enzyme that was crystallized and analyzed by single crystal X-ray diffraction methods was extracted from *Pseudomonas nautica* and purified under aerobic conditions in 2000.[22] The results of the measurement revealed the structure of the two different copper-containing active sites.

The structure of the Cu_A-center is almost similar to the one from cytochrome *c* oxidase.[23-24] The binuclear copper site is bridged by two cysteinato residues which leads to a very efficient electron delocalization in the oxidized $Cu^{1.5+}Cu^{1.5+}$-state. The coordination sphere of the copper ions is completed by one histidine residue and a methionine residue for the first copper ion and a tryptophane residue for the second copper ion, respectively (Figure 2.3).

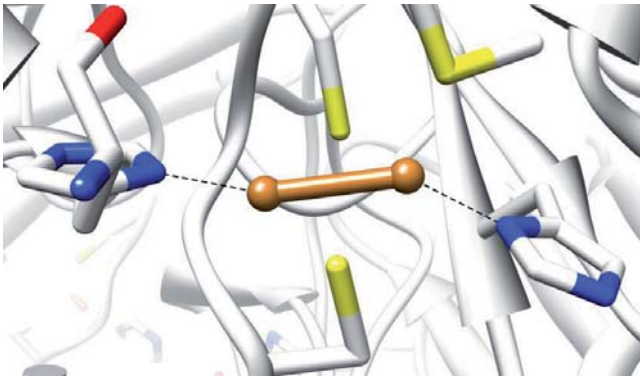


Figure 2.3: Cu_A-center of N_2OR from *Pseudomonas nautica*.[25]

Cu_A is a highly efficient electron transfer unit.[26] The distance of 2.5 Å between the two copper ions implied a copper-copper bond, which was proved *via* EXAFS spectroscopy.[27-28]

The Cu_Z^*-center comprises a μ_4-sulfido bridged tetranuclear copper cluster in the inactive, pink form (Figure 2.4).[25]

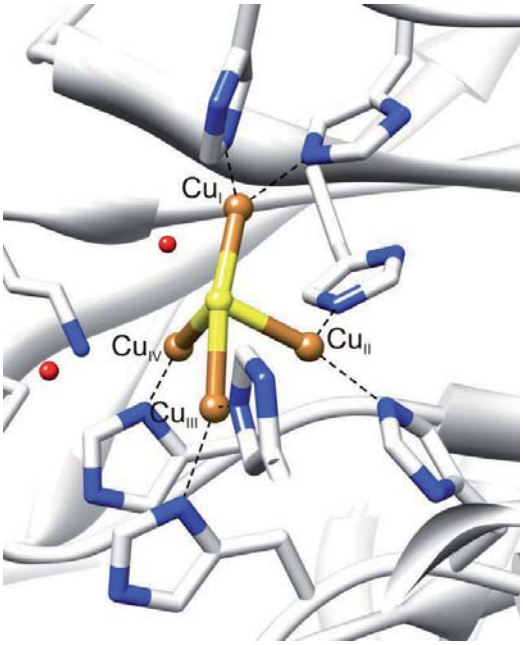


Figure 2.4: Cu_Z-center of N_2OR from *Pseudomonas nautica*.[25]

The Cu_4S moiety is coordinated by seven histidine residues as depicted in Figure 2.4. The nature of the molecule between the Cu_I and the Cu_{IV} ion could not be clearly identified[22, 25, 29] and was finally assigned as hydroxide or water molecule.[30]

The pink aerobic form of the enzyme has been studied over the last years to elucidate the catalytic cycle of N_2O reduction,[29, 31-33] but the detailed mechanism of the decomposition

of N_2O at the active site is not yet clarified. Theoretical studies suggested an initial μ-1,3-coordination of N_2O between the Cu_I and the Cu_{IV} ion of the cluster.[32] The redox-state of the active form was assumed to be all-cuprous and the bridging sulfido ligand was expected to facilitate electron delocalization over the whole unit after the two electron reduction process.[34]

The active purple form of N_2OR[35-36] is exclusively available under strict anaerobic conditions.[20, 37] Recently, the appendent crystal structure was published.[13] The structure exhibits a slightly different binding motif of the Cu_Z-site. An additional sulfide ligand has been incorporated between the Cu_I and the Cu_{IV} ions yielding a Cu_4S_2-motif (Figure 2.5). As in the previously reported structure of the pink N_2OR the copper ions are coordinated by seven histidine residues.

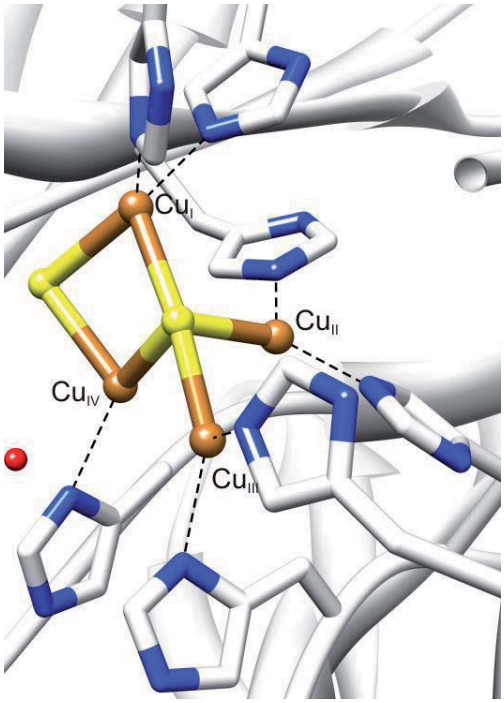


Figure 2.5: Structure of the recently discovered anaerobic Cu_Z-site of *Pseudomonas stutzeri*.[13]

The second bridging sulfido ligand seems to disappear in the oxic form. Presumably under aerobic conditions dioxygen can enter the inner sphere of the enzyme and might remove the coordinated sulfido ligand irreversibly. This is corroborated by the low activities of aerobically purified N_2OR.[34]

Additionally EINSLE *et al*. were able to obtain crystals suitable for X-ray diffraction of an enzyme-substrate adduct (Figure 2.6). In contrast to previous studies, they showed that N_2O is bound *side-on* above the Cu_{II} and Cu_{IV} ions and the central sulfido ligand.

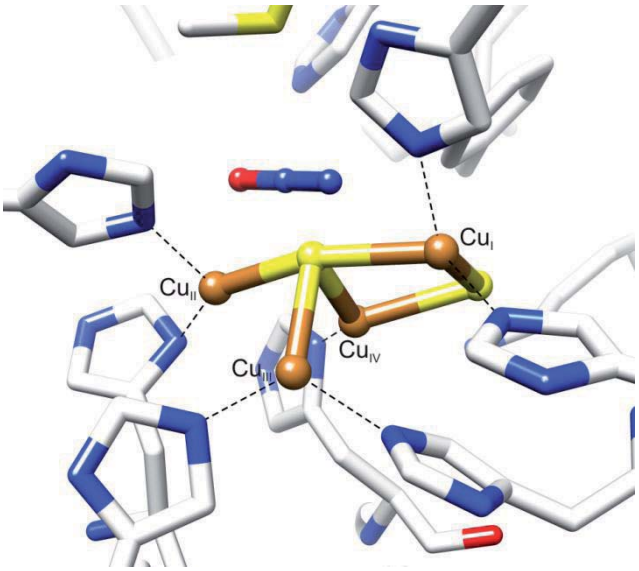


Figure 2.6: Substrate binding in N_2OR.[13]

These recent results push the investigations of N_2OR into a different direction. Spectroscopic data have to be re-evaluated under the new aspects.

2.3. Low-molecular weight models in bioinorganic chemistry

Metalloenzymes are involved in numerous processes that impact life and the environment. These enzymes are able to perform catalytic reactions with high selectivity and efficiency. Up to now the relationship between function and structure is not yet fully understood for most metalloenzymes.

In nature, the active site of proteins is surrounded by the protein cavity. Therefore spectroscopic investigations and studies about the fundamental steps of the reaction at the active center are very difficult. One possibility to get a deeper insight into these processes, is the synthesis of low molecular weight complexes that somehow mimic the active site of the targeted metalloenzyme. Detailed characterization and reactivity studies