Chapter 8

Summary, Conclusions & Outlook

8.1 Summary

8.1.1 Introduction

Chemical reactions in biological systems are catalyzed by a great variety of enzymes. Many of these enzymes contain copper, because of its ability to cycle between Cu^{II} and Cu^{I} oxidation states without difficulty.[1] The catalyzed reactions are very fast and efficient and to understand these processes, synthetic models are used to gain more insight into how these reactions take place within the enzyme and to see whether we can learn from them for other catalytic processes. Our interest was captured by the electron-shuttling Cu_A site which contains two copper ions bridged by two cysteine thiolate moieties and is found in cytochrome *c* oxidase and N_2O reductase.[2-4] Designing synthetic models for this active site is challenging, because of the redox reaction that commonly takes place upon addition of a thiolate compound to a Cu^{II} ion: 2 Cu^{II} + 2 LS^- → 2 Cu^{I} + LSSL. However, it has been reported that this redox reaction actually is an equilibrium, which can be influenced by ligand structure, solvent or the presence of chloride ions.[5-8] A schematic view of this equilibrium is given in Figure 8.1.

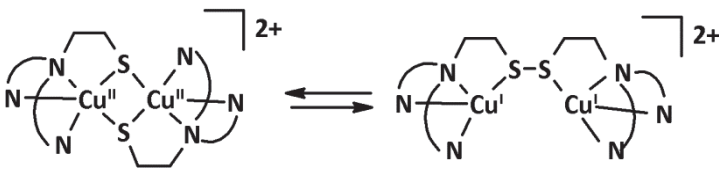

Figure 8.1: Schematic representation of the redox equilibrium between a Cu^{II} μ-thiolate complex and a Cu^{I} disulfide species.

In Chapter 1, an overview is given of different types of copper enzymes and their active sites. This is followed by a sum-up of the different synthetic analogs of the Cu_A site among which are a number of Cu^{II} μ-thiolate complexes. Subsequently different reported Cu^{II} μ-thiolate/Cu^{I} disulfide equilibria are discussed together with a similar cobalt-based equilibrium.

8.1.2 The Cu^{II} μ-Thiolate - Cu^{I} Disulfide Equilibrium

The equilibrium between the Cu^{II} μ-thiolate and Cu^{I} disulfide species can be controlled by inducing small ligand changes, adding chloride ions or changing the solvent. There is, however, limited understanding of why or when the Cu^{II} μ-thiolate complex forms. In Chapter 2, two new ligands **L^2SSL^2** and **L^4SSL^4** are introduced

which, together with three ligands from literature,[5] form two series of ligands with either increasing bulk on the pyridyl ring or an increasing number of (longer) ethylene bridges towards the pyridyl ring. The two new ligands both have the ability to form Cu^{II} μ-thiolate complexes but the complexes differ in their redox-conversion to the Cu^{I} disulfide species. When the ligand **L^2SSL^2** is combined with a Cu^{I} salt, it forms a Cu^{II} μ-thiolate complex in apolar solvents but a Cu^{I} disulfide species in polar solvents. The Cu^{II} μ-thiolate complex can be converted to a Cu^{I} disulfide complex by adding the polar solvent CH_3CN to the less polar CH_2Cl_2. Furthermore, we found that this equilibrium is temperature-dependent and can be reversibly switched at lower temperatures in methanol with the Cu^{I} species being the thermodynamically favored product at higher temperatures. The ligand **L^4SSL^4** forms a Cu^{II} μ-thiolate complex when reacted with a Cu^{I} salt in both polar and apolar solvents, but this Cu^{II} compound is irreversibly converted to the Cu^{I} disulfide complex by heating. So in the case of **L^4SSL^4**, the Cu^{II} μ-thiolate species is formed as the kinetic product whereas the Cu^{I} disulfide complex is the thermodynamic product. To gain insight in the energies that are involved in these equilibria and what causes the observed differences, DFT calculations were carried out on all the Cu^{II} thiolate and Cu^{I} disulfide complexes of the two ligand series. It was found that the Cu^{I} disulfide species is indeed lower in energy than the Cu^{II} μ-thiolate species, but that there is an increase in energy difference between the two states with increasing steric bulk or increasing number of ethylene bridges in the ligands. This implies that the energy of deformation of the ligand from its equilibrium structure to the geometry in the complex determines whether the Cu^{II} μ-thiolate complex forms. The position of the equilibrium between the two redox-isomeric species seems to be largely dependent on the activation barrier of going from the thiolate to the disulfide complex.

Another Cu^{II} μ-thiolate/Cu^{I} disulfide equilibrium with a very similar ligand was reported to be controlled by the presence of protons.[9] Isolation of a complex containing both Cu^{II} thiolate moieties and dissociated Cu^{I} ions directed us to consider the possibility that the proton-induced conversion of a Cu^{II} thiolate compound to a Cu^{I} disulfide species might actually result in dissociation of the ligand. Our investigations in this proton-induced conversion are described in Chapter 3. ^{1}H NMR and UV-vis measurements showed that the ligand dissociates from the copper ions after addition of 4 equiv of protons to the dinuclear Cu^{II} μ-thiolate species; a number of protons that corresponds to one proton per pyridyl group. However, from the experimental data it was not clear what happens after addition of two H^+. To clarify this, the protonation of these complexes was studied computationally with DFT. The calculations showed that indeed the pyridyl-nitrogen is protonated, but in addition

revealed that ligand dissociation is favored by more than 100 kJ mol^{-1} over conversion to a protonated Cu^{I} disulfide complex. The ligand dissociation reaction is schematically shown in Figure 8.2. A similar ligand dissociation reaction was shown experimentally to occur for a Cu^{I} disulfide complex.

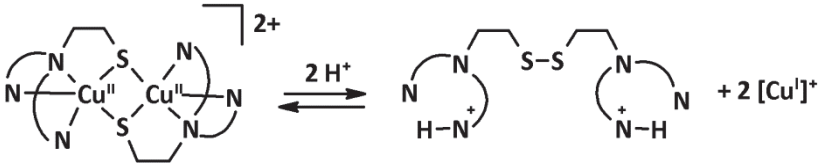

Figure 8.2: Schematic representation of the ligand dissociation that takes place after protonation of a Cu^{II} μ-thiolate complex.

8.1.3 Cu^{II} μ-Thiolate Oxidation

Many copper-containing proteins are involved in O_2 activation reactions and some of these copper enzymes contain sulfur ligands. It is therefore of interest to know how oxidation sensitive these enzymes are. Furthermore, thiols such as cysteine play an important role in biological reduction/oxidation chemistry via disulfide formation, but also via further oxidation to sulfenic, sulfinic and sulfonic acids.[10] In Chapter 4, our studies concerning the oxidation of a Cu^{I} thiolate complex are described. Isolation of a multinuclear Cu^{II}-Cu^{I} thiolate complex that is a potential intermediate in the oxidation reaction, together with UV-vis measurements, indicated that the oxidation proceeds via a Cu^{II} μ-thiolate complex, thus initially via oxidation of copper instead of direct oxidation of the thiolate ligands. The oxidation of the Cu^{I} thiolate species ultimately results in formation of Cu^{II} sulfinate and Cu^{II} sulfonate complexes that were isolated, analyzed and compared.

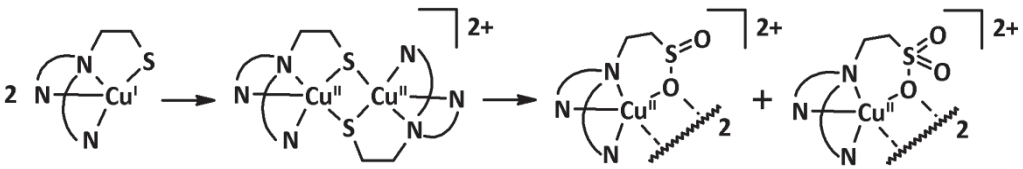

Figure 8.3: Schematic representation of the complete oxidation of a Cu^{I} thiolate complex to Cu^{II} sulfinate and Cu^{II} sulfonate complexes via formation of a Cu^{II} μ-thiolate species.

It was found that the sulfinate and sulfonate complexes are spectroscopically very similar, but electrochemically quite different. This difference is most likely caused by

the relative hardness of the sulfonate group that is not suitable for stabilization of the Cu^{I} oxidation state. A schematic overview of the oxidation process is given in Figure 8.3.

8.1.4 Small Ligand Changes Prevent Cu^{II} μ-Thiolate Formation

The Cu^{II} μ-thiolate complex provides a synthetic model for the Cu_A active site, but its synthesis is complicated by its conversion to a Cu^{I} disulfide complex. Small changes in the ligand structure can determine whether a Cu^{II} μ-thiolate or Cu^{I} disulfide complex is formed. The synthesis of three new disulfide ligands is described in Chapter 5, to analyze the effects on Cu^{II} μ-thiolate formation. The ligand **L^1SXSL^1** is similar to the ligand **L^{1*}SSL^{1*}** that was shown to form Cu^{II} μ-thiolate compounds, but is made more rigid by incorporation of an extra carbon-carbon bond. This ligand precludes Cu^{II} μ-thiolate formation, because of the large rearrangement necessary to allow for intermolecular Cu^{II} thiolate formation. The other ligands described in Chapter 5 have different electronic properties due to substitution of the pyridyl group by a pyrazole or 3,5-dimethylpyrazole moiety. Reaction of these pyrazole-based ligands with Cu^{I} salts resulted in formation of Cu^{I} complexes that are stable in air for days. The half-wave potentials of these three Cu^{I} complexes were determined and compared to the half-wave potential of a Cu^{I} complex that was formed by thermal conversion of a Cu^{II} μ-thiolate compound (Chapter 2). The comparison indicates that in order to allow formation of both Cu^{II} μ-thiolate and Cu^{I} disulfide species, a half-wave potential of roughly 0 V vs Ag/AgCl would be ideal. A number of Cu^{II} complexes obtained with the disulfide ligands and different counterions are also described in Chapter 5.

8.1.5 Similarities Between Copper-Thiolate and Copper-Oxygen Chemistry

A large part of biomimetic copper research reported in literature has been focused on aromatic ring oxidation of phenols and catechols, because these reactions are models for the activity of enzymes like tyrosinase and catechol oxidase.[1, 11] In Chapters 6 and 7 the reaction of copper complexes with phenols and catechol is described; the results reveal the similarities between copper-thiolate and copper-oxygen chemistry.

A mixture of a Cu^{I} complex with phenol was opened to air, which resulted in formation of a dark purple mononuclear Cu^{II} phenolate species. Its structure was determined by X-ray crystallography and described in Chapter 6. The formation of this species is interesting, because usually oxidation leads to ring oxygenation or oxidative phenol coupling.[12] Over time, the purple phenolate complex undergoes a

unique redox reaction to form a yellow Cu^{I} species and a phenolate radical, that reacts further to form a diaryl peroxide or an aromatic ether. This was confirmed by NMR and UV-vis spectroscopy and the appearance of a new Raman band. The rate of conversion is dependent on the concentration of the mononuclear species as well as the *para*-substituent on the phenyl group. A schematic representation of the conversion is given in Figure 8.4; the ligand that is used in this conversion is similar to the ligand used for Cu^{II} μ-thiolate formation. This redox reaction is analogous to the copper thiolate/disulfide redox reaction, only instead of converting 2 R-S^- to 2 R-$S^\bullet$ that reacts further to R-S-S-R, R-O^- is converted to R-$O^\bullet$.

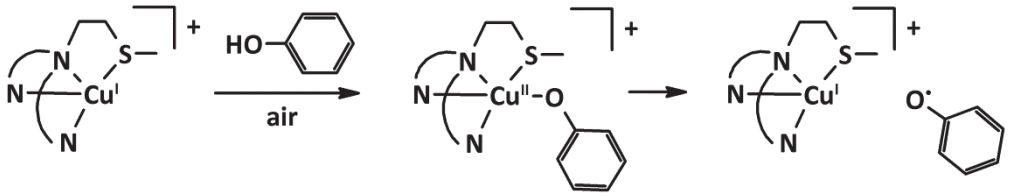

Figure 8.4: Schematic representation of the formation of a Cu^{II} phenolate species and its further redox conversion into a Cu^{I} complex and a phenolate radical.

To further investigate the similarities between the copper-oxygen and copper-sulfur chemistry, the catalytic oxidation of 3,5-di-*tert*-butylcatechol (3,5-DTBC) to 3,5-di-*tert*-butyl-*o*-benzoquinone (3,5-DTBQ) was investigated; the results are reported in Chapter 7. A large library of mononucleating and dinucleating ligands was synthesized and with these ligands [Cu^{II}(ligand)(Cl)](BF_4) complexes were formed to test in catalysis. For a number of these complexes crystal structures were obtained, that showed relatively large Cu…Cu distances for the dinucleating ligands. The catalytic oxidation of 3,5-DTBC occurred with higher turnover frequencies (TOFs) using the catalytic systems based on dinucleating ligands than those containing mononucleating ligands, confirming the need for two copper sites in catalysis. Furthermore, the ligands with the ability to form Cu^{II} μ-thiolate complexes displayed the highest TOFs; the highest initial TOF reached with such a system was 7800 h^{-1}. However, the oxidation of 3,5-DTBC became slower in time indicating catalyst degradation. These results again imply that there is a relation between thiolate formation with copper ions and their reaction with dioxygen. It can be hypothesized that the high activity is caused by the ability of these complexes to stabilize the Cu^{II}_2 μ-η^2:η^2-peroxide intermediate as it is similar to the Cu^{II} μ-thiolate complex. After all,

formation of the Cu^{II}_2 μ-η^2:η^2-peroxide species has been postulated to be the rate-determining step in the reaction.[11, 13]

8.2 Conclusions & Outlook

The equilibrium between Cu^{II} μ-thiolate and Cu^{I} disulfide species has been shown very sensitive to small changes in either ligand structure or reaction conditions. In this thesis, the energies involved in this equilibrium have been clarified to give a better view on why either one or the other species forms. Other aspects such as formation, protonation and oxidation of the Cu^{II} μ-thiolate complex have also been investigated yielding insight in the impact of different possible reactions occurring in biological systems, such as pH changes and oxidative enzyme degradation. However, it is difficult to determine whether these synthetic analogs are representatives for the active sites in metalloenzymes. Most of the reactions described in this thesis have been carried out in acetonitrile, which is polar, but not a protic solvent like water. Furthermore, acetonitrile stabilizes Cu^{I}, whereas H_2O generally leads to disproportionation of Cu^{I} salts into Cu^{II} and Cu^{0} when they are not stabilized by their ligands. Enzymes create an environment around the active site in which hydrogen bonding plays an important role, which is rather difficult to mimic in a small model system. Still, there are indications that the reactions that we investigated are similar to what actually may occur in the biological system. The ligand dissociation we observed after protonation of a Cu^{II} μ-thiolate species, has been postulated to occur similarly in the Cu_A site as well. It has been hypothesized that protonation of the Cu_A site induces dissociation of one of the equatorial histidine ligands, which might also lead to a change in valence state of the copper ions.[14-18] In this case the synthetic model does allow for a more detailed understanding of the natural active site. However, the reactivity of the synthetic models described in this thesis should not be used to draw conclusions about the biological system, but they can be used to support existing data about enzymatic structures and reactions.

The similarities between copper-thiolate and copper-oxide chemistry are demonstrated in Chapter 6 and 7. This is a relatively unexplored field of coordination chemistry. The conversion of a phenolate to a phenolate radical by oxidation with Cu^{II} ions is unprecedented and deserves further attention. The fact that sulfur and oxygen react in a similar way with copper and are interchangeable in some cases, allows for exploring other synthetic reactions with sulfur or oxygen-containing compounds. Furthermore, if the efficiency of 3,5-DTBC oxidation is indeed increased by better stabilization of the Cu^{II}_2 μ-η^2:η^2-peroxide intermediate by ligands that

support Cu^{II} μ-thiolate-type complexes, other types of oxidative catalysis might be worthwhile studying with these systems.

8.3 References

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