in addition, would lead to some very reactive and harmful intermediates.

In mitochondria the reduction of oxygen occurs through the action of cytochrome *c* oxidase. Electrons are transferred from reduced cytochrome *c*, presumably via a heme (cytochrome *a*) and a copper ion (Cu_A), to a binuclear center consisting of one heme (cytochrome a_3) and one copper ion (Cu_B). The individual steps of the reaction can be followed at sub-zero temperatures with optical and EPR spectroscopy. It seems that only the binuclear center is directly involved in the dioxygen reduction, and the initial steps after mixing dioxygen with the fully reduced enzyme are:

$$O_2 + a_3^{2+}Cu_B^{1+} \rightarrow a_3^{3+}{-}O^{-}{-}O^{-}{-}Cu_B^{2+} \xrightarrow[e^-]{H^+}$$

$$a_3^{4+}O^{2-}\cdots{}^{-}HOCu_B^{2+}$$

The structure of the last species is derived from its unusual EPR spectrum [1], which indicates the presence of a weak antiferromagnetic interaction between a Cu^{2+} ion and an $S = 1$ or 2 heme. Further support for this structure comes from ^{17}O hyperfine structure in experiments with isotopically enriched dioxygen.

In the reaction above, dioxygen is formally reduced in a process consisting of two two-electron transfers, thereby circumventing the O_2^- and OH· states. The catalytic cycle is completed by the transfer of three more electrons to the binuclear center, reducing the heme and the copper ion to the ferrous and cupric states, respectively.

Much less is known about the reverse reaction, the oxidation of water to dioxygen in the photosynthetic systems. Manganese ions most likely are involved, and so far the only paramagnetic intermediate detected suggests the presence of antiferromagnetically interacting Mn ions [2, 3]. However, from such observations and analogies with the cytochrome *c* oxidase reaction, a model can be proposed that again involves two two-electron transfers [4].

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Metallothionein: a Diamagentic Metal–Thiolate Cluster Protein

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Metallothionein (MT) is an ubiquitously occurring, extremely sulfur- and metal-rich protein (mol. wt. 6–7000) which plays a role in the metabolism and the detoxification of several essential and nonessential trace metals. All mammalian forms consist of a single polypeptide chain with a total of 61 amino acid residues out of which 20 are cysteines. Each molecule binds 7 bivalent metal ions, most commonly Zn(II) and Cd(II). All cysteine residues participate in metal coordination through formation of metal–mercaptide bonds. Their position in the polypeptide chain is preserved in all mammalian forms. Unique features are the –Cys–X–Cys– sequences (where X stands for an amino acid other than Cys) occurring 7 times along the chain.

MT is an elongated nonglobular protein with an axial ratio of about 6 [1, 2]. As documented by ^1H NMR and IR spectroscopy, it is a compact molecule with some secondary structure; however, it has not been crystallized as yet [3]. Hence, detailed structural information comes exclusively from spectroscopic studies. The electronic absorption spectrum is dominated by contributions from the electron transfer transitions of the metal–thiolate complexes superimposed upon the plain absorption spectrum of the polypeptide chain. The positions of the first electron transfer band are strongly metal-dependent. The locations displayed in the derivatives of MT containing Zn, Cd, Hg or Pb are in accordance with Jørgensen's optical electronegativity scale. They coincide very closely with the shifts predicted for tetrahedral tetrathiolate model complexes, thus suggesting the same metal coordination in MT [4]. This is corroborated by the spectroscopic properties of MT reconstituted with Co(II). The visible and near-IR absorption spectrum of the green-colored derivative shows, besides the Co(II)–thiolate electron transfer bands, d–d maxima at 600, 682 and 743 nm belonging to the $\nu_3[^4A_2 \rightarrow {}^4T_1(P)]$ transition and at 1230 nm belonging to the $\nu_2[^4A_2 \rightarrow {}^4T_1(F)]$ transition. These are diagnostic of distorted tetrahedral tetrathiolate high-spin Co(II) complexes, a conclusion further substantiated by the magnetic circular dichroism (MCD) and electron spin resonance (ESR) spectra [5]. Independent evidence for the tetrahedral structure is also available for Zn(II)–MT based on extended X-ray absorption fine structure (EXAFS) measurements [6] and for Cd(II)–MT based on perturbed angular correlation of gamma ray (PAC)

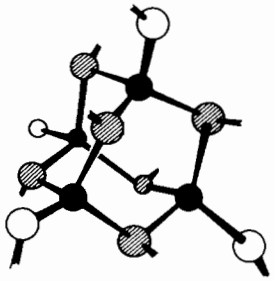

Fig. 1. Adamantane-type of metal–thiolate cluster proposed for metallothionein. The filled circles represent the metal, the empty circles terminal sulfur ligands, and the hatched circles the bridging sulfur ligands (from Ref. 3).

measurements using MT which contains the short-lived excited isomer ^{111m}Cd(II) [7].

The involvement of four sulfur ligands in the coordination of each metal ion is reconcilable with the overall $Me(II)_7(Cys^-)_{20}$ stoichiometry of the molecule only if the metal complexes are sharing some thiolate ligands, thereby forming metal–thiolate clusters. This conjecture is now also confirmed experimentally. Thus, the partitioning of the metal-coordinating ligands into bridging and non-bridging (terminal) thiolate ligands is manifested in a substantial broadening of the sulfur core electron binding energy profile in the X-ray photoelectron spectrum (ESCA) of MT as compared to those of elemental sulfur or monothiols [3]. Direct proof for clustering of the metal centers comes from the demonstration of metal–metal interactions by magnetic resonance spectroscopy. Thus, in ^{113}Cd-enriched MT ^{113}Cd NMR resonances are split into multiplets by ^{113}Cd–^{113}Cd scalar coupling [8] and in fully substituted Co(II)–MT the ESR-resonances are largely suppressed due to antiferromagnetic spin-canceling. This latter effect is also confirmed by magnetic susceptibility measurements [9]. By monitoring the changes in ESR amplitude and in magnetic susceptibility attending the stepwise incorporation of Co(II) into the protein, it is moreover possible to follow the building-up of the cluster structure. This process is biphasic. Up to binding of 4 equivalents of Co(II) the paramagnetic signals increase as expected for magnetically noninteracting high-spin complexes. However, upon further addition of Co(II), these features are lost again, signalling the transition to the magnetically interacting oligonuclear structures [9].

This two-step mode of metal-binding in which separate Co(II) tetrathiolate complexes are formed first is compatible with the model of two separate metal–thiolate clusters in MT deduced from ^{113}Cd NMR homonuclear decoupling experiments [10] and from proteolytic cleavage studies [11] as well as from a ^{113}Cd NMR study on cluster formation in progress in this laboratory. However, the actual steric organization of these clusters remains to be determined. An attractive structure consistent with all spectroscopic information at hand is the cage-like adamantane decahedron built up of thiolate units of tetrahedral symmetry (Fig. 1). The remarkable simplicity of the spectra and the unique capacity of MT to accommodate stoichiometrically different paramagnetic and diamagnetic metal ions, *i.e.*, Ni(II), Co(II), Zn(II), Cd(II), Hg(II), Pb(II) and Bi(III), and to force them into environments of tetrahedral microsymmetry could be optimally accounted for by such a model or a variation of it. The need for these regular bioinorganic structures in MT would explain the remarkable preservation of the positions of all cysteine residues throughout mammalian evolution.

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Second Coordination Sphere Influences on Heme Electronic Structure and Reactivity in Hemoproteins

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A large variety of oxygen-binding hemoproteins with quite different functions share the common iron protoporphyrin IX prosthetic group axially ligated by a histidyl imidazole. To the degree that the members of this class of proteins differ in reactivities and electronic/magnetic properties, they