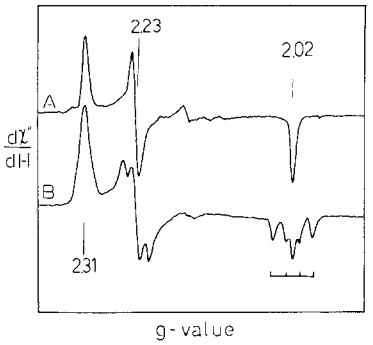

Fig. 1. EPR spectra of *D. gigas* hydrogenase enriched (B) and unenriched (A) ^{61}Ni enzyme. The EPR experiments were performed with a Bruker 200-series EPR-spectrometer. EPR data was taken at ~100 K, using 100 KHz modulation frequency and 9.256 GHz microwave frequency. Adapted from ref. 9.

processes. It was shown to be a structural component of the hydrogenases isolated from *Desulfovibrio gigas* [1, 2], *Desulfovibrio desulfuricans* (ATCC 27774) [3], *Desulfovibrio desulfuricans* (Norway strain) [4], *Methanosarcina barkeri* [5], *Methanobacterium thermoautotrophicum* [6] and *Chromatium vinosum* [8]. With the exception of the last one, they were demonstrated to contain EPR nickel redox dependent signals. As an example, *D. gigas* hydrogenase exhibits rhombic EPR signals, with g-values 2.31, 2.23 and 2.02 (see Fig. 1). Using isotopic reconstitution by ^{61}Ni (nuclear magnetic moment I = 3/2), the EPR signal was proven to arise from a nickel species [9]. The same types of experiments were reported for *M. thermoautotrophicum* [6] and *D. desulfuricans* (ATCC 27774) hydrogenases [3].

A detailed EPR study on the oxidation–reduction transition of the EPR detectable species in the presence of reductants (dithionite and hydrogen) indicates [1, 2]:

(a) The reduction of the Ni EPR active species is an one-electron process (possibly associated with the redox couple Ni(III)–Ni(II)).

(b) No evidence was found so far for exchangeable protons in the vicinity of the nickel center in the oxidized (native) state. However, hydrogen reduced samples originate a different EPR rhombic Ni signal, which may represent an active transient species occuring during the activation of hydrogen molecules [9]. Thus, it is attractive to propose the presence of a hydride intermediate in analogy with nickel catalysts involved in hydrogenation processes [10].

(c) Although the determined mid-point redox potential (–220 mV) is more negative than that expected for nickel compounds [11] it is still more positive than that of the substrate couple H_2/H^+. The value determined was shown to be pH dependent [2].

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B13

Intermediates in the Reduction of Dioxygen by Cytochrome *c* Oxidase and in the Photosynthetic Water Oxidation

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The cycle of oxygen in the biosphere is totally dominated by two processes, photosynthetic oxidation of water to dioxygen on the one hand and reduction of dioxygen to water on the other. There is one problem common to the mechanism of these two reactions; at least at some point, electrons are carried one at a time, whereas the dioxygen–water reaction requires a total of four electrons. In some cases thermodynamic requirements effectively prevent four consecutive one-electron transfers, which,

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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B14

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)