$oxac^{2-}$, while in hydration the base attacks the keto carbon atom through an H_2O molecule, $-C(=O)-CH_2- + HOH\cdot\cdot B \rightleftharpoons -C(O^-)(OH)-CH_2- + BH^+$. In either case, an oxyanion is formed at the 2-carbon atom.

Metal ion complex formation has a relatively small effect on the rate constants for acid catalyzed enolization, suggesting that there is little interaction between the metal ion and the site for proton attack, the keto group oxygen atom. In contrast, the rates along the base catalyzed pathways are strongly influenced by a metal ion and exhibit increases that appear more dependent on the nature of the catalyst than on the reaction mode. The presence of Mg(II) induces increases of three orders of magnitude in the rates of OH^- catalyzed enolization and hydration, while the H_2O catalyzed pathway for hydration and the tertiary amine catalyzed pathways for both reactions undergo rate increases of 20–30 fold. A charge effect is apparent in these figures, but, especially in the case of OH^-, the metal ion must interact with, and stabilize the intermediate oxyanions in ways similar to that by which decarboxylation is promoted.

Rates of ketonization of enolpyruvate are also being investigated. Consistent with the charge differences acid catalysis is slower, and base catalysis is faster with enolpyruvate than with $oxac^{2-}$. Complexing metal ions show very strong cooperativity with base catalysts. Here again, the metal ion seems to function as a stabilizer of the intermediate oxyanion.

The coordinating abilities of metal ions are paramount in their causing activation barriers to be lowered in these model reactions. However, recent evidence obtained in a detailed study of enzymic decarboxylation suggests that an enzyme may not utilize this function of a metal ion, but may achieve greater catalytic efficacy through other mechanisms, such as proton transfer.

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Active Site of Copper Amine Oxidases

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Copper amine oxidases catalyse the oxidative deamination of biological amines according to the following equation:

$$RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + H_2O_2 + NH_3$$

It should be recalled that another class of amine oxidases was described. They contain FAD as a prosthetic group, but will not be discussed here.

All copper amine oxidases have molecular weight higher than 100,000: some of them appear to contain two equivalent subunits which are probably not linked by disulfide bridges [1, 2]. All these enzymes contain at least two copper atoms and one carbonyl group per molecule. The nature of the carbonyl cofactor is a controversial issue: many data suggest that it could be identified as pyridoxal-5-phosphate [1].

Electron spin resonance (ESR) spectra show that the two copper atoms are in an equivalent environment and in the cupric state [3, 4]. The spectrum is typical of Cu(II) in a complex of tetragonal symmetry [3]. The use of ^{15}N labeled putrescine demonstrated that the substrate is not directly bound to copper [3]. Magnetic resonance studies [3, 5] and the comparison of the absorption and ESR spectra of the bovine enzyme with a model complex [6] suggest that in these enzymes at least two nitrogen atoms and 2 oxygen (from water) are coordinated around the copper ion.

When copper amine oxidases were treated with phenylhydrazine, 1 carbonyl group/mol was titrated [1] and an enzymatically inactive irreversible adduct was formed: its formation requires the presence of cupric copper [7].

After treatment with phenylhydrazine the bovine plasma enzyme shows changes in the ESR parameters of only one copper [7], indicating that the two copper sites are not identical. The non-identity of copper sites was demonstrated also in the pig plasma enzyme by magnetic studies [5]. The copper atoms however, become equivalent after treatment with strong ligands like N_3^- and CN^- [5]. These results indicate a functional significance of the difference between the two copper atoms. Since the enzyme molecule contains two non identical copper ions and only one carbonyl group, these findings suggest that only one of the two coppers has a role in the substrate oxidation. If the second prosthetic group is pyridoxal-5-phosphate, one of the copper ligands could be the nitrogen of the pyridine ring [1]. The substrate may bind the carbonyl cofactor and copper may have a catalytic role in oxygen activation [7].

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Low-temperature Magnetic Circular Dichroism Spectra of Metallo-proteins

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As an optical probe of the metal centres in proteins magnetic circular dichroism (MCD) spectroscopy has two features of value in biochemistry. First, the MCD spectra of paramagnetic centres are temperature dependent increasing in magnitude as the temperature is lowered, whereas those of diamagnetics are temperature independent. A study of the magnetic field and temperature dependence of the MCD signal down to 1.5 K and up to 5 Tesla enables the state of magnetisation of a paramagnet to be determined. From such a magnetisation curve it is possible to determine the spin (S) of the ground state and, in favourable case, the effective ground state g-factors [1]. Thus identification is possible of optical absorption bands which belong to species detectable by EPR spectroscopy. Furthermore, paramagnets which are invariably invisible to EPR spectroscopy, that is, even-electron spin systems or odd-electron spin paramagnets with $M_S > \pm\frac{1}{2}$ as the lowest energy Kramers doublets, can be investigated. Secondly, the MCD spectra of vibrational transitions are orders of magnitude weaker than those of electronic transitions. Hence electronic states in the MCD spectra of metalloproteins can be detected out to wavelengths as long as 2500 nm. This enables metal localised d–d states and metal–ligand charge-transfer (CT) states to be located. The energies of these states can be sensitive indicators of the coordination environment of a metal centre in a protein. When applied together these two features of MCD spectroscopy make it a powerful tool in unravelling the electronic structures of metal centres especially those in proteins with more than one centre [2]. We describe several examples drawn from our own work of the last few years.

A ferromagnetic haem–copper pair in cytochrome c oxidase. The near infrared MCD spectrum of cyanide-inhibited bovine cytochrome *c* oxidase shows three bands, at 790 nm, 1564 nm and 1946 nm which can be assigned, on the basis of their MCD magnetisation properties, to Cu_A^{2+}, the EPR detectable copper ion, cytochrome a^{3+}, the low-spin EPR detectable haem, and cytochrome a_3^{3+} bridged to Cu_B^{2+} [3]. The latter apparently comprise a pair of metal ions bridged, probably linearly, by CN^- and ferromagnetically coupled to give a ground state spin, S = 1, with a zero-field splitting of ~10–20 cm^{-1} leaving a pair of levels, $M_S = \pm 1$, virtually degenerate and lower in energy than the $M_S = 0$ component [4]. An orbital coupling scheme will be presented to rationalise this unique behaviour and also to account for the unusually long wavelength, 1946 nm, of the haem charge-transfer (porphyrin → Fe^{III}) band. The optical properties of this metal pair, which lie at the active site of the enzyme, have been explored in a number of derivatives. The functional implications of these features are discussed by Dr. C. Greenwood in his paper.

EPR-silent states of iron–sulphur proteins. The low temperature MCD spectra of iron–sulphur clusters in paramagnetic oxidation states are highly structures giving excellent means of identifying cluster type [2]. A number of examples will be given to illustrate the identification of cluster type and oxidation state in complex proteins. The MCD characterisation of proteins containing [3Fe-xS] centres will be described and the transformation of 3Fe to 4Fe clusters and *vice versa* will be shown [5]. A discussion of cluster type and properties in the proteins nitrogenase and hydrogenase will also be given [6].

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