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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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