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L22 CHARACTERISATION OF THE OXIDISED P-CLUSTERS IN THE MoFe PROTEIN OF *Klebsiella pneumoniae* NITROGENASE BY MCD SPECTROSCOPY

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Crystallographic studies of nitrogenase have confirmed earlier spectroscopic work from Hagan and Pierik [1] showing that this enzyme contains an unusual iron-sulphur cluster, called the P-centre, in which a pair of cuboids, [4Fe-4S], are linked by bridging sulphide ion and a pair of thiolate ligands. The P-cluster functions as a storage and transfer site for electrons en route from the electron donor, the iron protein, to the site of di-nitrogen reduction, a hetero-atom iron-molybdenum-sulphur cluster. The magnetic and optical properties of the P-cluster have been studied using magnetic circular dichroism (MCD) spectroscopy in order to characterise its electronic properties. The MCD spectra of the MoFe protein, *Klebsiella pneumoniae* Kp1, at various poised potentials between -460 mV and +200 mV (vs. NHE) were measured at 4.2K and 5 Tesla. The P-cluster is oxidised at a mid-point potential of $E_n = -300$ mV by a two-electron process ($n = 2$) from a diamagnetic state to an integer spin state characterised by an EPR signal at $g \sim 12$ detected in the parallel mode, and by an intense MCD spectrum [2]. The MCD magnetisation properties, monitored at several wavelengths over a range of fields and temperature, have been successfully analysed for the first time. The EPR and MCD magnetisation results can be fitted to a ground electronic state, $S = 4$, with a negative, axial zero field splitting parameter $D = -2$ cm⁻¹.

1. A.J. Pierik, H. Wassink, H. Haaker and W.R. Hagen, *Eur.J.Biochem.*, 212, 51-61 (1993).
2. M.K. Johnson, A.J. Thomson, A.E. Robinson and B.E. Smith, *Biochim.Biophys.Acta*, 671, 61-70 (1981).