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J09 SOME PROPERTIES OF THE [2Fe-2S] RIESKE-TYPE FERREDOXIN AND ITS MUTANTS FROM BENZENE DIOXYGENASE IN *PSEUDOMONAS PUTIDA* ML2.

P. Unalkat^a, O. Hatzfeld^b, T. A. Link^b, H-M. Tan^c, R. Cammack^a, J. R. Mason^a.

^a Centre for the Study of Metals in Biology & Medicine, King's College London, Campden Hill Road, London, W8 7AH, United Kingdom.

^b Universitätsklinikum Frankfurt, ZBC, Therapeutische Biochemie, Theodor-Stern Kai 7, 60596 Frankfurt-am-Main, Germany.

^c Department of Microbiology, National University of Singapore, Singapore.

EPR, ESEEM and ENDOR have been used in order to probe the [2Fe-2S] cluster of this small (~ 12 kDa) [2Fe-2S] protein. EPR of the wild-type (WT) protein shows characteristic g-values at $g_z = 2.026$, $g_y = 1.890$, $g_x = 1.834$, consistent with other "Rieske-type" proteins.

ESEEM reveals the presence of strong hyperfine features in the 1-10 MHz region, the number and magnitude of which indicate couplings to two nitrogenous ligands. These nitrogens are thought to arise from the histidine ligands to the [2Fe-2S] cluster. ENDOR has been used to measure hyperfine couplings of protons to the [2Fe-2S] cluster, which probably arise from cysteine and histidine side-chains.

The EPR spectra of four of its mutants, G46A, G57A, L64F and E61T are essentially unchanged with the notable exception of E61T, showing a broadening and a shift of g_x to 1.809.

The mid-point potentials (E_m) were determined by EPR, Cyclic Voltammetry and Circular Dichroism using an Optically Transmitting Thin Layer Electrode. The WT protein showed $E_m = -150 \pm 5$ mV. Mutations of amino acids adjacent to the histidine ligands of the cluster showed an increase in E_m to -125 mV and -105 mV for the L64F and G46A mutants respectively, whereas a mutation adjacent to a co-ordinating cysteine (E61T) showed $E_m = -185$ mV, becoming more "plant-type" in its behaviour. These differences may be interpreted in terms of changes in the cluster environment.