

**M011 THE ROLE OF DIVALENT METAL IONS AS CO-REPRESSORS FOR THE REPRESSOR PROTEIN FUR: A STUDY OF THE Co(II) COMPLEX\***

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Ferric uptake regulation (FUR) is a 17 KDa molecular weight repressor protein involved in iron uptake regulation in *Escherichia. Coli* [1]. Fur dimer binds a 19 bp DNA sequence "iron box" using Fe(II) ion, *in vivo*, as co-repressor. *In vitro*, when the Fe(II) concentration reaches a certain level, the Fur dimer is activated by complexing to two Fe(II) ions to form a fur metal ion complex which binds to the DNA[2].

It is established that Co(II) activates the Fur protein to bind the DNA *in vitro*[2]. Electronic absorption studies enabled the assignment of a distorted tetrahedral environment around the Co(II) ( $\lambda_{max} \approx 715, 670, \text{ and } 530 \text{ nm}$ ). Equilibrium studies gave evidence of the association of up to 6 Co(II) ions per Fur monomer and presence of a weak site,  $K_d=600 \mu\text{M}$ , and stronger site  $K_d=60 \mu\text{M}$ . The epr parameters ( $g=1.9, g=4.9$ ) of the Co(II) fur complex were consistent with the electronic absorption spectra, i.e. both gave evidence of the presence of Co(II) in a distorted tetrahedral environment. The complex of the mutant Fur C92S, C95S, and the C92SC95S with Co(II) helped to characterize the position of ligating sites to the metal ion on the Fur sequence. The binding of Fur Co(II) complex to an oligonucleotide representing the "iron box" altered the Co(II) environment indicating an increase in axiality. This was evident in epr parameters ( $g=4.93, g=3.8, \text{ and } g=1.9$ ).

The type of ligands provided by the Fur to the metal ion and the likely role of the metal ion in DNA binding are discussed.

1. A. Bagg and J.B. Neilands, *Micorobiol. Rev.*, 509, 51(4), (1987).
2. V. deLorenzo, S. Wee, M. herrero, and J.B. Neilands, *J. Bacteriol.*, 2624, 169(6) (1987).