Stabilizing a [4Fe-4S] Cluster to Oxygen: The Influence of a Hydrogen Bond Donor at Postion 28 in the FNR Transcription Factor.

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The oxygen-sensing transcription factor FNR is found in the facultative anaerobe *Escherichia coli*. Dimerization and DNA binding of wild type FNR are coupled to the incorporation of a [4Fe-4S] cluster, which is ligated by cysteine residues at positions 20, 23, 29, and 122. In the presence of O_2, this cluster degrades to a [2Fe-2S] cluster, which results in a conformational change that renders the protein inactive. Previous research has shown that the Leu28His mutant FNR protein has an oxygen stable [4Fe-4S] cluster. Several more amino acid substitutions have been made at residues adjacent to the cysteine ligands. The *in vivo* activity of these mutant FNR proteins has been evaluated by β-galactosidase assays under aerobic and anaerobic conditions. Most of the mutant proteins retained similar activity to that of the wild type protein, with significant activity under anaerobic condition and little activity under aerobic conditions. However, replacement of Leu28 with Lys, Arg or Gln resulted in proteins that are active under aerobic conditions. These results suggest that a substitution of a hydrogen bond donating residue at position 28 stabilizes the cluster to oxygen. The Leu28Lys FNR protein has been isolated and preliminary characterization of its [4Fe-4S] cluster by absorption spectroscopy shows that like the Leu28His FNR protein, the [4Fe-4S] cluster in Leu28Lys FNR is stable in the presence of oxygen. The [4Fe-4S] cluster of the mutant proteins are currently being characterized in order to give us a better understanding of how a hydrogen bond donating residue at position 28 helps to stabilize the cluster in the presence of oxygen.