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The Analysis of Amino Acid Substitutions at Positions ASP22 of FNR

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THE ANALYSIS OF AMINO ACID SUBSTITUTIONS AT POSITIONS ASP22 OF FNR

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The FNR protein is an oxygen-sensing transcription factor in the facultative anaerobe, Escherichia coli. In wild-type FNR, a [4Fe-4S] cluster in incorporated into the protein. This results in a protein that dimerizes and can bind to specific sites in the DNA. In the presence of oxygen, this cluster degrades causing a conformational change that renders the protein inactive. The Fe-S cluster is ligated by cystein residues at positions 20, 23, 29, and 122. Our work focuses on the effects on protein activity of amino acid substitutions at position Asp22. We studied the aerobic and anaerobic activity of the mutations Asp22Ala, Asp22Ser, Asp22Trp, Asp22Asn and Asp22Gly using Beta-galactosidase assays. Of these mutations Asp22Gly has been previously reported to be aerobically active, and it was suggested that this substitution stabilized the cluster to oxygen. Currently, under the low protein concentrations of our Beta-galactosidase assays, none substitutions have shown aerobic activity. We have also isolated Asp22Ala and Asp22Gly FNR protein. The Asp22Ala FNR protein has been characterized using absorption spectroscopy, which shows that the [4Fe-4S] cluster is not stable to the presence of oxygen. Further research will include isolation of other Asp22 FNR proteins as well as performing Beta-galactosidase assays with a higher concentration of protein.