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Examining the Role of Asparagine 564 in *E. coli* MetH by Site-Directed Mutagenesis

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Cobalamin-dependent methionine synthase (MetH) is a large, modular protein that catalyzes the methyl transfer from methyl-tetrahydrofolate (Fol) to homocysteine (Hcy) to form methionine and tetrahydrofolate. MetH uses a cobalamin cofactor as an intermediate methyl carrier in the reaction. The primary focus of this project was the Fol domain. This domain activates methyltetrahydrafolate so that it can transfer a methyl group to cob(I)alamin (Cob) to form methylcobalamin (MeCob). The Asparagine 564 amino acid resides in the active site of the Fol domain and was previously hypothesized to stabilize the N5 atom of the pterin ring on methyltetrahydrofolate by forming a through-water hydrogen bond to the nitrogen atom. This interaction, observed in the crystal structure of MetH, is believed to be important for methyltetrahydrofolate activation. For this project, Asp564 was mutated to a glutamic acid residue (N564E). It was hypothesized this change would increase substrate activation due to the longer and more acidic nature of the side chain. Asp564 was also mutated to an aspartic acid residue (N564D). Primers were designed with these mutations, generating the mutant plasmids, then transformed into XL10 Gold Ultracompetent cells. The mutant MetH proteins were then purified and used in Fol kinetics assays using the wild type MetH as a control. The N564D mutant protein had a k_{cat} of 850 $M^{-1}s^{-1}$ for the Fol assay, compared to 550 $M^{-1}s^{-1}$ for wild type MetH. The N564E MetH, had a k_{cat} calculated lower than either the wild type or N564D MetH protein. These unexpected results may indicate that the acidic amino acid at this position is better at stabilizing the charge on the N5 during catalysis, but that the increase in length of the carbon chain from D to E leads either to crowding or non-ideal hydrogen bond geometries in the active site.