Ph Dependence Studies of the DNA Glycosylase Mutty

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Deoxyribonucleic acid (DNA) is the molecule that contains the instructions for making proteins and directing all the activities of the cell. Damage to DNA is thus very harmful to the cell and leads to diseases such as cancer. All organisms, therefore, have several genes that code for DNA repair enzymes. These enzymes catalyze the reactions that repair the lesions of damaged DNA.

The E.coli DNA repair enzyme MutY is an adenine glycosylases which removes adenine (A) when it is mispaired with deoxyguanosine (G) or 7,8-dihydro-8-oxo-2'-deoxyguanosine (OG). This prevents transversion mutations that would occur after subsequent replications of the DNA. This is very significant to the cell because such mutations can be carcinogenic and/or cytotoxic. In fact, it has been shown that mutations in the human homologue of MutY lead to increased incidence of colon cancer.

Experiments to study the pH dependence of the enzyme are useful to gain insight into its mechanism. Wild type MutY shows a pH dependence with the substrate strand G:A (see Appendix). It is proposed that since wild type MutY loses activity at both low and high pH, that the mechanism is based on acid/base catalysis. This suggests that the rate-determining step of the mechanism could be the catalytic chemical step. In order to further probe this hypothesis, mutated forms of the enzyme are

However, a truncated form of MutY called Stop225 (S225) has been shown to have a pH dependence with OG:A in that it loses catalytic activity at high pH values (see Appendix). This was expected because S225 does not show any activity preference toward G:A versus OG:A, whereas wild type does. The result indicates that an important catalytic amino acid residue of S225 must be protonated for catalysis to occur, but is becoming deprotonated at high pH. However, it could also mean that the rate-determining step of S225 is not the catalytic step, but rather a conformations change that is affected by a change in pH.

Another enzyme, S120K, was also studied. This enzyme has the serine residue (S) at position 120 replaced by site-directed mutagenesis to lysine (K). First, the total concentration of the enzyme was determined using a Bradford assay with BSA as a standard. Next, its percent activity was determined to be 22.6% by active site titration experiments.

S120K was studied using G:A as substrate, and it was shown that, unlike wild type, it does not have a pH dependence. This signifies that the serine residue is responsible for the pH dependence in wild type. However, it could also mean that the rate-determining step was changed in S120K to be a step that is independent of the effects of pH. It is difficult to draw concrete conclusions from the amount of data collected thus far. Future experiments to further our insight of the