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Glycosylases utilize "stop and go" motion to locate DNA damage

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Oxidative damage to DNA results in alterations that are mutagenic or even cytotoxic. Base excision repair is a mechanism that functions to identify and correct these lesions, and is present in organisms ranging from bacteria to humans. DNA glycosylases are the first enzymes in this pathway and function to locate and remove oxidatively damaged bases, and do so utilizing only thermal energy. However, the question remains of how these enzymes locate and recognize a damaged base among millions of undamaged bases. Utilizing fluorescence video microscopy with high spatial and temporal resolution, we have observed a number of different fluorescently labeled glycosylases (including bacterial FPG, NEI, and NTH as well as mammalian MutyH and OGG). These enzymes diffuse along DNA tightropes at approximately 0.01+/- 0.005 μm2/s with binding lifetimes ranging from one second to several minutes. Chemically induced damage to the DNA substrate causes a $\sim$ 50% reduction in diffusion coefficients and a $\sim$ 400% increase in binding lifetimes, while mutation of the key "wedge residue" – which has been shown to be responsible for damage detection - results in a 200% increase in the diffusion coefficient. Utilizing a sliding window approach to measure diffusion coefficients within individual trajectories, we observe that distributions of diffusion coefficients are bimodal, consistent with periods of diffusive motion interspersed with immobile periods. Utilizing a unique chemo-mechanical simulation approach, we demonstrate that the motion of these glycosylases can be explained as free diffusion along the helical pitch of the DNA, punctuated with two different types of pauses: 1) rapid, short-lived pauses as the enzyme rapidly probes DNA bases to interrogate for damage and, 2) less frequent, longer lived pauses that reflect the enzyme bound to and catalytically removing a damaged base. These simulations also indicate that the wedge residue is critical for interrogation and recognition of damage, and thus enzymes missing this residue diffuse faster. Similarly, chemically induced damage increases the frequency with which the enzymes encounter damaged bases, resulting in slower diffusion.