DNA-Mediated Charge Transport as a Probe of MutY/DNA Interaction†

Elizabeth M. Boon,‡ Mary Ann Pope,§ Scott D. Williams,§ Sheila S. David,*§ and Jacqueline K. Barton*‡

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, and Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

Received November 19, 2001

Revised Manuscript Received March 13, 2002

Abstract:

MutY is an *Escherichia coli* DNA repair enzyme that binds to 8-oxo-G:A and G:A mismatches and catalyzes the deglycosylation of the mismatched 2'-deoxyadenosine. We have applied DNA-mediated charge transport to probe the interaction of MutY with its DNA substrate. Oligonucleotides synthesized with a tethered rhodium intercalator and guanine doublets placed before and after the MutY binding site are used to assay for base flipping activity by MutY. On the basis of this assay, we find no evidence that MutY uses progressive base flipping as a means to find its binding site; protein binding does not perturb long-range DNA charge transport. DNA-mediated charge transport can be utilized to promote protein-DNA cross-linking from a distance. Long-range oxidation of 8-oxo-G within the MutY binding site using tethered rhodium intercalators promoted cross-linking and yielded information on MutY side chains that interact with this base. On the basis of photooxidative cross-linking of the wild type but not K142A mutant, it is evident that, within the protein complex, lysine 142 makes important contacts with 8-oxo-G.