Supporting Information for:

Recognition of the Nonpolar Base 4-Methylindole in DNA by the DNA Repair Adenine Glycosylase MutY

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Experimental Section

Materials. The plasmid containing the *mutY* gene, pKKYEco, and *E. coli* strains JM101 *mutY*$^-$ were kindly provided by M. L. Michaels and J. H. Miller as described previously.[1-3] Standard 2'-deoxynucleotide-β-cyanoethyl phosphoramidites were purchased from Applied Biosystems and the OG phosphoramidite was purchased from Glen Research. The 2'-deoxy-2'-fluoroadenosine phosphoramidite was kindly provided by Greg Kamilar and Dr. Peter Beal (University of Utah). All substrate 2'-deoxyoligonucleotides were synthesized on an Applied Biosystems automated oligonucleotide synthesizer model 392. The 5'-end labeling was performed with T4 polynucleotide kinase purchased from New England Biolabs and [γ-^{32}P] ATP from Amersham Life Sciences. Labeled oligonucleotides were purified using Probe Quant G-50 spin columns from Amersham Pharmacia. All PCR reagents were purchased from Boehringer Mannheim. Bovine serum albumin (BSA) and Bradford reagents were purchased from BioRad. All other reagents were of analytical purity and purchased from Fisher, USB, or Mallinckrodt Baker, Inc. PCR reactions were performed in a GeneAmp PCR system 2400. Storage Phosphor autoradiography was performed using a Molecular Dynamics Storm 840 PhosphorImager using ImageQuant (version 4.2a) software.

Protein overexpression, purification, and active site concentration of WT MutY and Stop 225. MutY was overexpressed and purified as described previously[4] with minor modification.[5] For Stop 225, site-directed mutagenesis was performed using a PCR based method similar to that described previously.[3] The stop codon and a *Pst*I restriction site were introduced using the appropriate primer (5'-gtg cgc tcc tgc agc gtc tat ttc ggt ttt ttg-3'). Stop 225 was overexpressed and purified as described.[5] The percent active enzyme concentrations of WT or Stop 225 was determined using an active site titration method modified for MutY.[6] The percent active concentrations based on the total protein concentration determined by Bradford of WT MutY and Stop 225 was 42% and 37%, respectively.

DNA substrate preparation. The following DNA duplexes containing a mispair at the position **X:Y** were prepared after automated synthesis and ion-exchange HPLC purification of each single stranded oligonucleotide:

(5'-CGATCATGGAGCCAC**Y**AGCTCCCGTTACAG-3')**.**(3'-GCTAGTACCTCGGTG**X**TCGAGGGCAATGTC-5'), with **Y**= 2'-deoxyguanosine (G) or 7,8-dihydro-8-oxo-2'-deoxyguanosine (OG), and **X**= 2'-deoxyadenosine (A), 2'-deoxy-2'-fluoroadenosine (FA), 2'-deoxytubercidin (Z) or 4-methylindole β-deoxynucleoside (M). The **Y**-containing strand was ^{32}P-5'-end-labeled and annealed to

1.5 molar excess of the complementary sequence in a buffer containing 150 mM NaCl, 20 mM Tris-HCl, pH 7.6, 10 mM EDTA. For active site titration determinations, only 3 to 5% of the A-containing strand was 5'-end labeled for accurate substrate concentration determination. Annealing was facilitated by heating the mixture to 90 °C and then slowly cooling to 25 °C for 4-6 hours.

Equilibrium-dissociation constant (K_d) determination. Gel retardation assays[7] were performed as described previously.[8] In the case of reactions with substrate duplexes containing OG and using WT enzyme, the final concentration of the duplex was 10 pM. With all other duplexes and experiments using Stop 225, the final concentration of the duplex was 20 pM. K_d was determined by fitting the data from a Storage Phosphor autoradiogram (percent bound substrate vs. log[MutY]) with the equation for one-site ligand binding using the program GraFit version 4.06 (from Erithacus). The reported K_d values are corrected for the activity of the enzyme as determined by the active site titration method.[6] In addition, each reported value is an average of at least four separate experiments. A representative plot of percent bound substrate vs. log[Stop 225] for a duplex containing an OG:M central mispair is shown in Figure A.

Melting Temperature (T_m) determination. Optical melting studies were performed as described previously.[9] Each 11-mer oligonucleotide was synthesized and purified as described above, and the following duplexes were prepared: (5'-GAGCT**OG**GTGGC-3')**.**(3'-CTCGA**X**CACCG-5') where X=A, M, or Z. Each solution containing a 1:1 ratio of the complimentary oligonucleotides in a buffer containing 10 mM $KHPO_4$, 1 M NaCl, and 1 mM EDTA was heated to 100°C and slowly cooled to 25 °C to facilitate annealing. The samples were degassed with argon and filtered through a 0.45 μm filter into a 1 cm path length quartz cell with a Teflon stopper. Extraction of melting temperatures (T_m) from the experimental data (absorbance vs. temperature) was accomplished using the standard assumption of a two-state transition. A representative melting curve for a duplex containing an OG:M central mispair is shown in Figure B.

References

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Fig. A. Representative plot of percent bound DNA determined from quantitation of gel retardation experiments as a function of Stop 225 concentration. The Stop 225 concentration is shown on a logarithmic scale. The K_d value for Stop 225 and a 30 base pair duplex containing a central OG:M mispair determined from this plot is 1.2 nM.

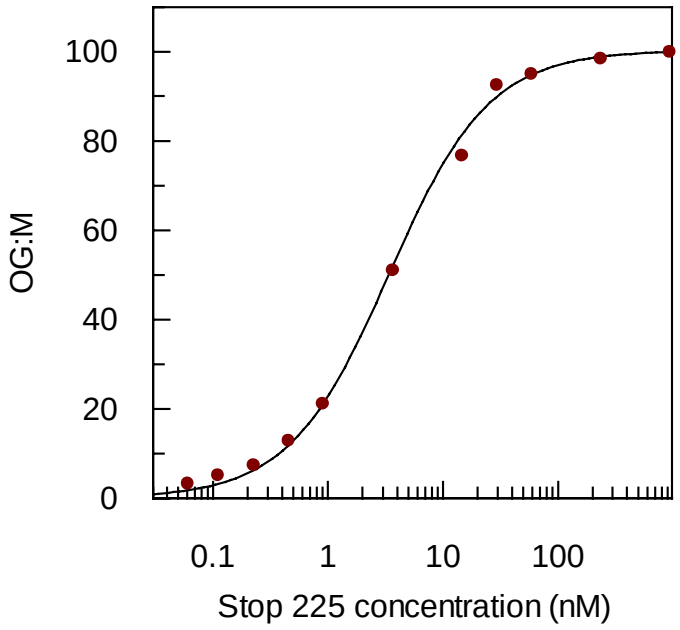

Fig. B. Representative melting curve of an 11 base pair duplex used in the optical melting studies. The raw data, the absorbance plotted as a function of temperature, was adjusted for the slopes of the single- and the double-stranded regions. The melting curve is plotted as α vs. temperature, where α = % double-stranded DNA. The T_m determined in this particular experiment was 49 °C.