(5'*S*)-8,5'-Cyclo-2'-deoxyguanosine is a strong block to replication, a potent pol V-dependent mutagenic lesion, and is inefficiently repaired in *Escherichia coli*

SUPPORTING INFORMATION

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Running title: Cyclo-dG mutagenesis and repair in *E. coli*

Materials and Methods

Materials

[γ-^{32}P] ATP was from Du Pont New England Nuclear (Boston, MA). *Eco*RV restriction endonuclease, T4 DNA ligase, T4 polynucleotide kinase, uracil DNA glycosylase and exonuclease III were obtained from New England Bioloabs (Beverly, MA). pMS2 plasmid was a gift from M. Moriya (SUNY, Stony Brook, NY).

The *E. coli* strains used were AB1157 [F$^-$ *thr-1 araC14 leuB6*(Am) Δ(*gpt-proA*)*62lacY1 tsx-33 supE44*(AS) *galK2*(Oc) *hisG4*(Oc) *rfbD1 mgl51 rpoS396*(Am) *rpsL31*(Str^r) *kdgK51 xylA5 mtl-1 argE3*(Oc) *thi-1*], pol II$^-$ (AB1157 but *polB*Δ*1*::Ω Sm-Sp), pol IV$^-$ (AB1157 but Δ*dinBW2*::cat), GW8017 (AB1157 but *umuDC595*::cat), and pol II$^-$/pol IV$^-$/pol V$^-$ (AB1157 but *polB*Δ*1*::Ω Sm-Sp *dinB umuDC595*::cat). All *E. coli* strains were provided by G. Walker (MIT, Cambridge, MA).

Methods

Synthesis and characterization of oligonucleotides. The *S*-cdG -modified oligonucleotides 5'-GTGCG*TGTTTGT-3', containing the DNA sequence of *p53* codons 272-275 in which the lesion was located in codon 273, was synthesized and characterized as reported (*1*). Unmodified oligonucleotides were analyzed by MALDI-TOF MS analysis, which gave a molecular ion with a mass within 0.005% of theoretical, whereas adducted oligonucleotides were analyzed by ESI-MS in addition to digestion followed by HPLC analysis.

Construction and characterization of pMS2 vectors containing a single *S*-cdG. The single stranded pMS2 shuttle vector, which contains its only *Eco*RV site in a hairpin region, was prepared as described (*2*). The pMS2 DNA (58 pmols, 100 μg) was digested with a large excess of *Eco*RV (300 pmol, 4.84 μg) for 1 h at 37^o C followed by room temperature overnight. A 58-mer scaffold oligonucleotide was annealed overnight at 9^o C to form the gapped DNA. The control and lesion

containing oligonucleotides were phosphorylated with T4 polynucleotide kinase, hybridized to the gapped pMS2 DNA, and ligated overnight at 16^{o} C. Unligated oligonucleotides were removed by passing through Centricon-100 and the DNA was precipitated with ethanol. The scaffold oligonucleotide was digested by treatment with uracil DNA glycosylase and exonuclease III, the proteins were extracted with phenol/chloroform, and the DNA was precipitated with ethanol. The final construct was dissolved in 1 mM Tris-HCl-0.1 mM EDTA, pH 8, and a portion was subjected to electrophoresis on 1% agarose gel in order to assess the amount of circular DNA.

Transformation in *E. coli* and analyses of progeny. The control and *S*-cdG construct were used to transform *E. coli* cells (*3*), and transformants were analyzed by oligonucleotide hybridization. Oligonucleotide probes containing the complementary 15-mer sequence were used for analysis (*4*). Two 14-mer left and right probes were used to select phagemids containing the correct insert, and transformants that did not hybridize with both the left and right probes were omitted. Any transformant that hybridized with the left and right probes but failed to hybridize with the 15-mer wild-type probe were subjected to DNA sequence analysis. Lesion bypass efficiency was calculated by comparing the transformation efficiency of the *S*-cdG construct with that of the control, whereas mutation frequency (MF) was calculated on the basis of hybridization and sequence analysis.

Substrate Construction and Protein Purification for UVRABC Incisions. DNA substrates of 51 bp containing *S*-cdG, *S*-cdA, and C8-dG-AP adducts were constructed as previously described (*5*). Briefly, a modified 12-mer, GTGCXTGTTTGT (X denotes the modified nucleotide), was ligated with a flanking 20-mer (GACTACGTACTGTTACGGCT) and a 19-mer (GCAATCAGGCCAGATCTGC) oligonucleotides at the 5'- and 3'-end, respectively. The 20-mer was terminally labeled with ^{32}P. The ligation product was purified by urea-PAGE running under

denaturing conditions. Following purification, the substrate was annealed to the corresponding complementary strand, and then purified on an 8% native polyacrilamide gel.

UvrA, UvrB, and UvrC proteins were overexpressed in *E. coli* then purified as previously described (*6*). Bio-Rad Protein Assay was used to determine the protein concentration under the recommended manufacturer's procedures.

Nucleotide Excision Assay. The 5'-terminally labeled DNA substrates were incised by UvrABC as previously described. Briefly, the DNA substrates (2 nM) were incubated in the UvrABC reaction buffer (50 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 5 mM DTT) at 37°C in the presence of UvrABC (UvrA, 10 nM, UvrB, 250 nM, and UvrC, 100 nM). Aliquots were collected in a time-course dependent manner at 0, 5, 10, 15, and 20 min. Enzymes were inactivated by heating to 95°C for five minutes. The products were then denatured with formamide loading buffer and heating to 95°C for five minutes, followed by quick chilling on ice. The products were then analyzed by electrophoresis on a 12% polyacrylamide sequencing gel run under denaturing conditions with TBE buffer.

Quantification of Incision Products. The radioactivity was measured in a quantitative manner using the Fuji FLA-5000 Image Scanner and Multi Gauge V3.0 software. The percentage of DNA incised (in fmol) by UvrABC was calculated based on the total molar amount of DNA used in each reaction and the total radioactivity as compared to the percentage of radioactivity of the incision products. At least three independent experiments were performed for determination of the rates of incision.

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References

1. Romieu, A., Gasparutto, D., and Cadet, J. (1999) Synthesis and characterization of oligonucleotides containing 5',8-cyclopurine 2'-deoxyribonucleosides: (5'R)-5',8-cyclo-2'-deoxyadenosine, (5'S)-5',8-cyclo-2'-deoxyguanosine, and (5'R)-5',8-cyclo-2'-deoxyguanosine, *Chem Res Toxicol 12*, 412-421.
2. Raychaudhury, P., and Basu, A. K. (2011) Genetic Requirement for Mutagenesis of the G[8,5-Me]T Cross-Link in Escherichia coli: DNA Polymerases IV and V Compete for Error-Prone Bypass, *Biochemistry 50*, 2330-2338.
3. Malia, S. A., Vyas, R. R., and Basu, A. K. (1996) Site-specific frame-shift mutagenesis by the 1-nitropyrene-DNA adduct N-(deoxyguanosin-8-y1)-1-aminopyrene located in the (CG)3 sequence: effects of SOS, proofreading, and mismatch repair, *Biochemistry 35*, 4568-4577.
4. Colis, L. C., Raychaudhury, P., and Basu, A. K. (2008) Mutational specificity of gamma-radiation-induced guanine-thymine and thymine-guanine intrastrand cross-links in mammalian cells and translesion synthesis past the guanine-thymine lesion by human DNA polymerase eta, *Biochemistry 47*, 8070-8079.
5. Yang, Z., Colis, L. C., Basu, A. K., and Zou, Y. (2005) Recognition and incision of gamma-radiation-induced cross-linked guanine-thymine tandem lesion G[8,5-Me]T by UvrABC nuclease, *Chem Res Toxicol 18*, 1339-1346.
6. Luo, C., Krishnasamy, R., Basu, A. K., and Zou, Y. (2000) Recognition and incision of site-specifically modified C8 guanine adducts formed by 2-aminofluorene, N-acetyl-2-aminofluorene and 1-nitropyrene by UvrABC nuclease, *Nucleic Acids Res 28*, 3719-3724.