Supporting Information for World Wide Web Edition

We failed to separate the isomeric oligodeoxynucleotides 5'-GAGCCAACCTG[(-)G*]CTCTGA and 5'-GAGCCAACCTG[(+)G*]CTCTGA (upper strands of duplexes (+)G_2^* and (−)G_2^*) by reversed-phase HPLC techniques to a high purity. Instead, we took advantage of the markedly slower electrophoretic mobilities of the oligodeoxynucleotide duplexes bearing (+)-*trans*-*anti*-B[*a*]P-N^2-dG adducts as compared to those of the (-)-*trans*-*anti*-B[*a*]P-N^2-dG adducts (*1*-*3*), to separate the (+)G_2^* and the (−)G_2^* duplexes from one another. Thereafter the unmodified strands 5'-TCAGAGCCAGGTTGGCTC were separated from the modified oligodeoxynucleotides 5'-GAGCCAACCTG[(-)G*]CTCTGA or 5'-GAGCCAACCTG[(+)G*]CTCTGA by 20% denaturing PAGE. These strands, as well as the 5'-GAGCCAACCT[(-)G*]GCTCTGA and 5'-GAGCCAACCT[(+)G*]GCTCTGA strands, were separately loaded onto denaturing 20% polyacrylamide gel to determine their mobilities. Each sample exhibited a single band indicating that the samples were of acceptable purity (Figure 1S). The modified oligodeoxynucleotides (each of the upper strands in oligodeoxynucleotide duplexes (+)G_1^*, (−)G_1^*, (+)G_2^*, and (−)G_2^*) moved more slowly than the corresponding unmodified oligodeoxynucleotides.

Table 1S. Thermodynamic parameters of helix-coil transition of B[*a*]PDE-modified DNA duplexes

Designation	Oligodeoxynucleotide duplex[a]	T_m, °C[b]	h, %[c]
U	5'-GAGCCAA**CCTGG**CTCTGA 3'-CTCGGTT**GGACC**GAGACT	70.3	16
(−)G_1^*	5'-GAGCCAA**CCT[(-)G*]G**CTCTGA 3'-CTCGGTT**GGA———C-C**GAGACT	65.0	15
(+)G_1^*	5'-GAGCCAA**CCT[(+)G*]G**CTCTGA 3'-CTCGGTT**GGA———C-C**GAGACT	65.5	15
(−)G_2^*	5'-GAGCCAA**CCTG[(-)G*]**CTCTGA 3'-CTCGGTT**GGAC—C—-**GAGACT	67.8	13
(+)G_2^*	5'-GAGCCAA**CCTG[(+)G*]**CTCTGA 3'-CTCGGTT**GGAC—-C—-**GAGACT	67.1	14

[a] – (-)G* is (-)-*trans*-*anti*-B[*a*]P-N^2-dG and (+)G* is (+)-*trans*-*anti*-B[*a*]P-N^2-dG.

[b] – accuracy of T_m determination is $\pm$ 0.5 degrees;C_{ds} 2 μM, buffer D, λ 260 nm.

[c] – hyperchromicity values was calculated according to equation: $h = \frac{A_{260}^{85^0} - A_{260}^{25^0}}{A_{260}^{85^0}} \cdot 100$,

where $A_{260}^{85^0}$ and $A_{260}^{25^0}$ are absorbances at 260 nm of oligodeoxynucleotides mixture at 85^0 and at 25^0C respectively.

The hyperchromicities for B[*a*]PDE-modified oligodeoxynucleotide duplexes (+)G_1^*, (−)G_1^*, (+)G_2^*, and (−)G_2^* ranged from 13-15%, which were not too different from the hyperchromicity observed in the case of the unmodified duplex U.

FIGURE LEGENDS

Figure 1S. Denaturing 20% PAGE of purified single-stranded ^{32}P-labeled 18-mer oligodeoxynucleotides: 5'-TCAGAGCCAGGTTGGCTC , 5'-GAGCCAACCTGGCTCTGA , 5'-GAGCCAACCT[(-)G*]GCTCTGA, 5'-GAGCCAACCT[(+)G*]GCTCTGA, 5'-GAGCCAACCTG[(-)G*]CTCTGA, 5'-GAGCCAACCTG[(+)G*]CTCTGA.

Figure 2S. Direct titration of fluorescein-labeled FAM-U duplex with M.EcoRII, studied by means of the fluorescence polarization method. The solid line represents a best fit to a simple binding isotherm. The concentration of the FAM-U is 50 nM. It is significantly higher than the value of K_d expected for the M.EcoRII/AdoHcy/FAM-U complex (the K_d for a M.EcoRII/AdoHcy/(unmodified 14-mer oligodeoxynucleotide duplex) was about 5 nM (*4*)). Thus, each protein molecule added to the reaction mixture is expected to bind to the oligodeoxynucleotide duplex. The value of P increases with increasing protein concentration leveling off at a value of P ≈ 0.30, characterizing the P value of the ternary complex M.EcoRII/AdoHcy/FAM-U. The point where the two asymptotic lines intersect corresponds to M.EcoRII concentration 475 nM. As the concentration of the M.EcoRII active form at this point is equal to the concentration of the FAM-U duplex (50 nM), the fraction of the enzyme bound is 11%.

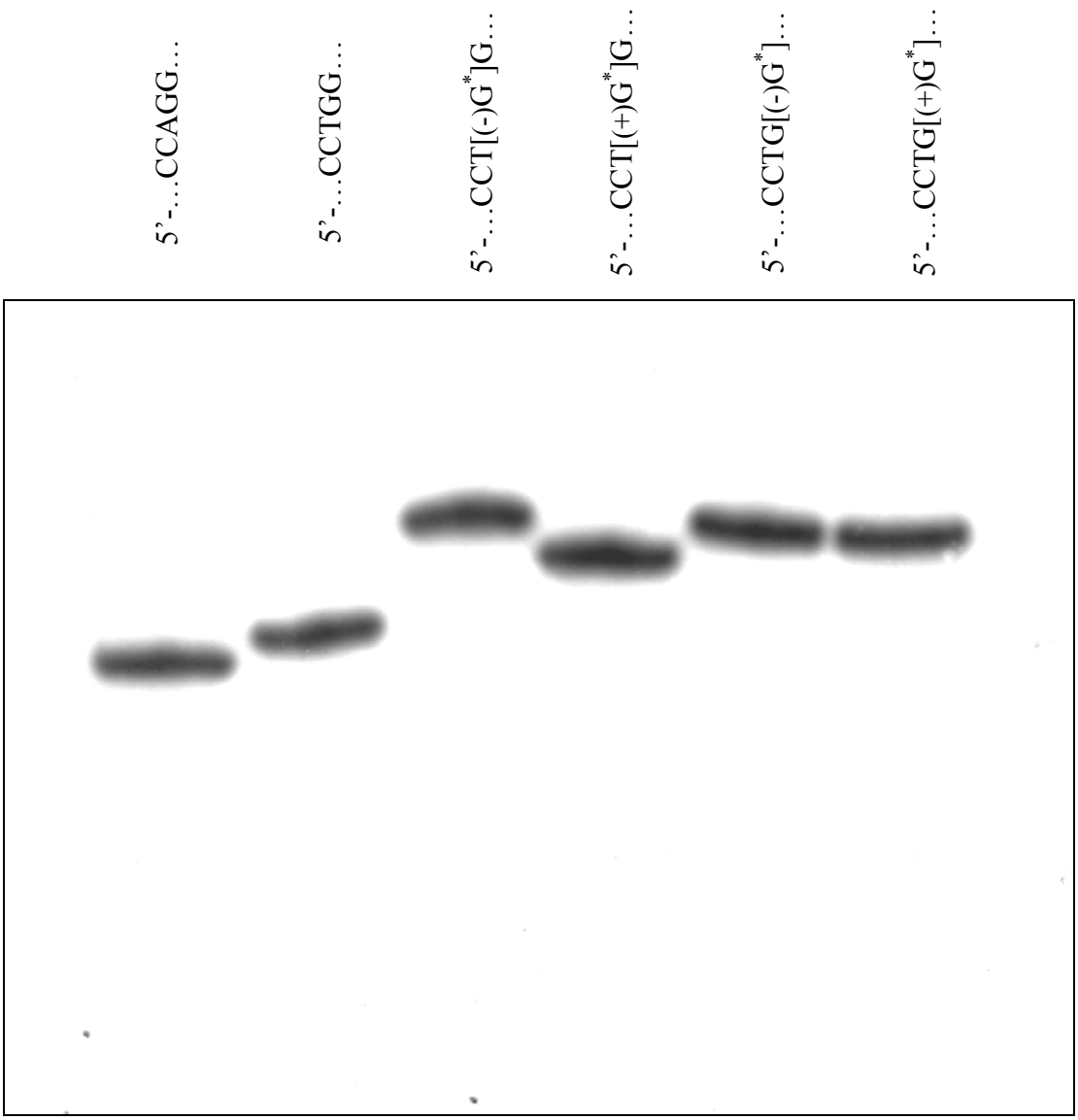

Fig. 1S

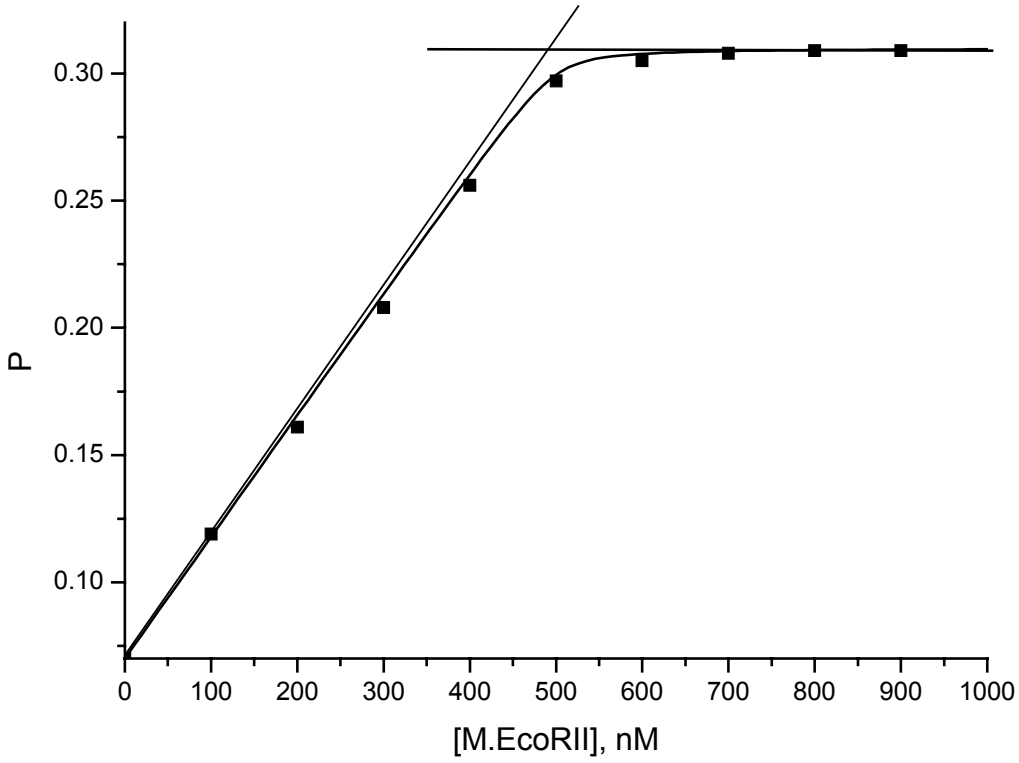
P
0.30
0.25
0.20
0.15
0.10
0
100
200
300
400
500
600
700
800
900
1000
[M.EcoRII], nM

Fig. 2S

1. Huang, X., Kolbanovskiy, A., Wu, X., Zhang, Y., Wang, Z., Zhuang, P., Amin, S., and Geacintov, N. E. (2003) Effects of base sequence context on translesion synthesis past a bulky (+)-*trans*-*anti*-B[*a*]P-N^2-dG lesion catalyzed by the Y-family polymerase pol κ, *Biochemistry 42*, 2456-2466.

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3. Huang, X., Colgate, K. C., Kolbanovskiy, A., Amin, S., and Geacintov, N. E. (2002) Conformational changes of a benzo[*a*]pyrene diol epoxide-N^2-dG adduct induced by a 5'-flanking 5-methyl-substituted cytosine in a (Me)CG double-stranded oligodeoxynucleotide sequence context, *Chem. Res. Toxicol., 15*, 438-444.

4. Subach, O. M., Khoroshaev, A. V., Gerasimov, D. N., Baskunov, V. B., Shchyolkina, A. K., and Gromova, E. S. (2004) 2-Pyrimidinone as a probe for studying the EcoRII DNA methyltransferase-substrate interaction, *Eur. J. Biochem. 271, 2391-2399*.