One-Electron Oxidation of DNA: Thymine versus Guanine Reactivity

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SUPPORTING INFORMATION

EXPERIMENTAL PROCEDURES

T4 polynucleotide Kinase (T4 PNK) was purchased from New England Biolabs. DNA oligomers and anthraquinone (AQ) containing complementary oligomers were synthesized on an Expedite 8909 DNA synthesizer in our lab. Nucleotide phosphoramidites are obtained from Glen Research/ChemGenes and used as received. The extinction coefficients of the oligomers were calculated using an online biopolymer calculator, and their concentrations were determined from the absorbance at 260 nm. An adenine is substituted for the anthraquinone group in the extinction coefficient calculation. The oligonucleotides were purified by means of reversed phase HPLC on a Hitachi preparative HPLC system using a Dynamax C18 column. Purified oligomers were desalted and characterized by mass spectroscopy. UV melting and cooling curves were recorded on a Cary 1E spectrophotometer equipped with a multicell block, temperature controller, and sample transport accessory. CD spectra were recorded on a JASCO spectropolarimeter.

Preparation of Radiolabeled DNA.

The oligomers were radiolabeled at the 5′-end using γ-^{32}P ATP and T4 PNK enzyme. A 5 μL sample of desired single stranded DNA was incubated with 1 μL of γ-^{32}P [ATP] and 2 μL of T4 Kinase in a total volume of 20 μL at 37 °C for ca. 45 min. After incubation, the DNA sample was suspended in a denaturing loading dye and was purified on a 20% nondenaturing polyacrylamide gel. The desired DNA band was excised from the gel and eluted with 800 μL of elution buffer (0.5 M NH_4OAc, 10 mM $Mg(OAc)_2$/1.0 mM of EDTA/0.1% SDS) at 37 °C for 12 h. The DNA was precipitated from the

supernatant by addition of ~600 μL of cold ethanol and 2 μL of glycogen. The mixture was vortexed, placed on dry ice for about 2 hours, and centrifuged at ~10 000g for 30 min- 45 minutes. The supernatant was removed, and the residual DNA was washed twice with 100 μL of 80% ethanol and air-dried. Suitable volumes of water were added for further experimentation.

UV Irradiation and Cleavage Analysis.

Samples for irradiation were prepared by hybridizing a mixture of unlabeled (5.0 μM) and radiolabeled (~10000 cpm) oligonucleotides with complimentary AQ-linked DNA in pH 7.0 10mM sodium phosphate and 2mM $MgCl_2$ buffer solution. $MgCl_2$ is added to enhance the thermal stability of DNA sequences, especially AT rich sequences. Hybridization was achieved by heating the samples at 90 °C for 10 min, followed by slow cooling to room temperature for at least three hours. Samples were irradiated at ca. 30 °C in microcentrifuge tubes in a Rayonet Photoreactor (Southern New England Ultraviolet Co., Barnsford, CT) equipped with 350 nm lamps. The irradiation times can vary for each sequence and typically single-hit conditions are maintained for further analysis of DNA strand cleavage. A time course study of irradiation of the DNA oligomers allows us to optimize the irradiation times for achieving single-hit conditions. After irradiation, the samples were precipitated once with cold ethanol (100 μL) and 2 μL of glycogen. The precipitated samples were washed twice with 100 μL of 80% ethanol, dried, and treated with 100 μL of 1 M piperidine at 90 °C for 30 min. After evaporation of piperidine and co-evaporation with water, the samples were dissolved in denaturing loading dye and subjected to 20% 19:1 polyacrylamide gel electrophoresis. The gels were dried, and the cleavage sites were visualized by autoradiography. Strand Cleavage ratios for each TT/ GG step are obtained by dividing the individual damage by total damage obtained in the strand. Quantification of cleavage bands was performed on a Fuji phosphorimager.

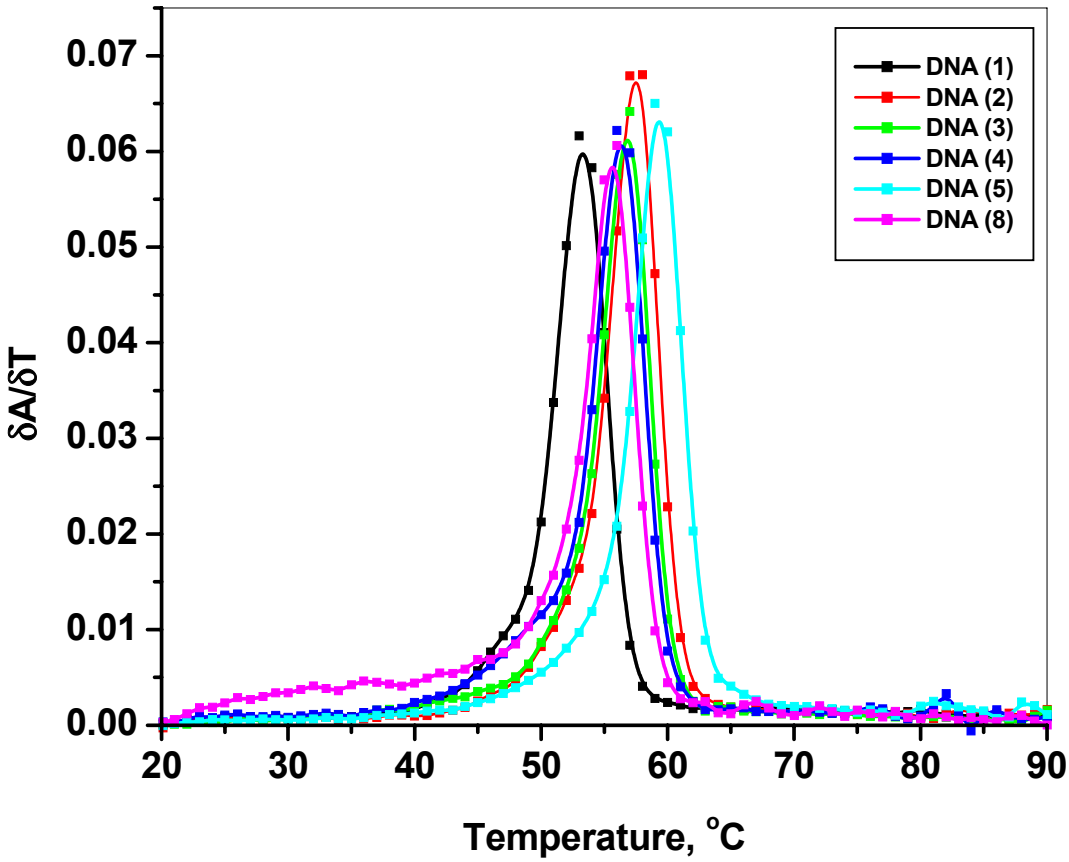

Figure S1: First derivative melting temperature profiles of DNA (**1**), (**2**), (**3**), (**4**), (**5**) & (**8**). The Melting Data for all the DNA sequences is given in Table S1.

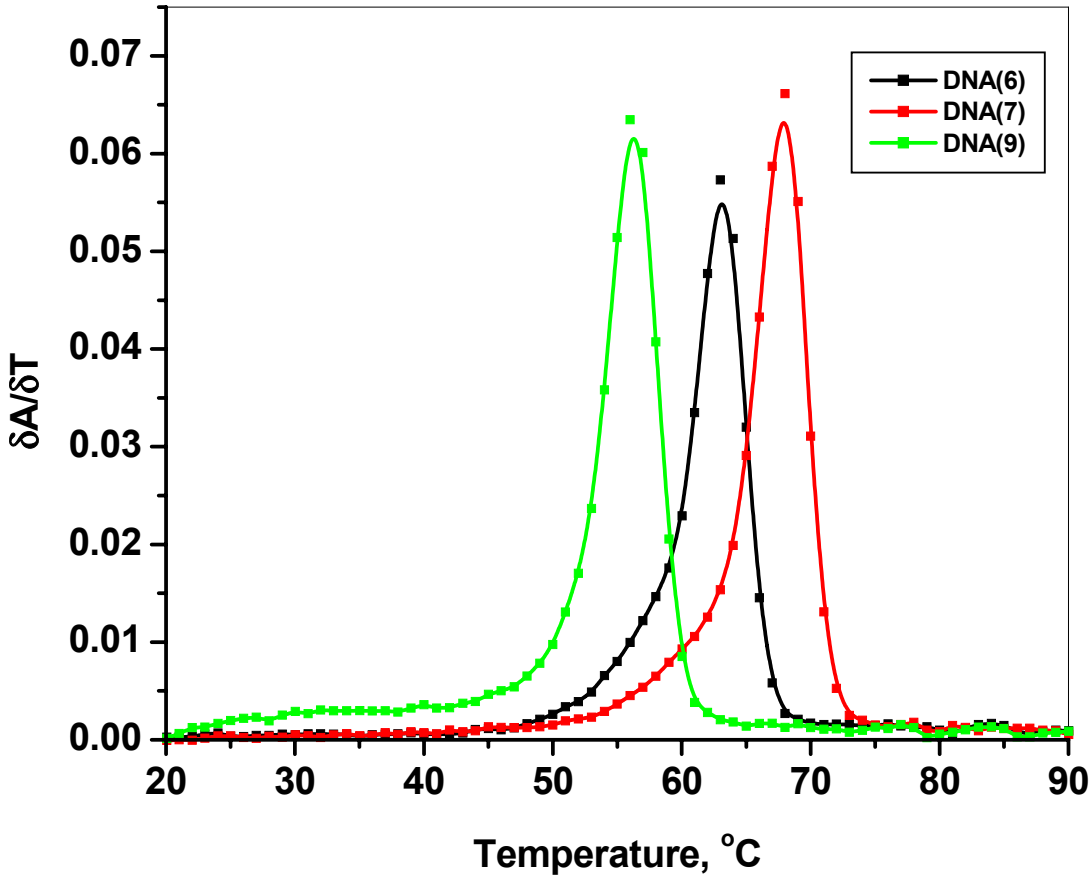

Figure S2: First derivative melting temperature profiles of DNA (**6**), (**7**), (**9**). The Melting Data for all the DNA sequences is given in Table S1.

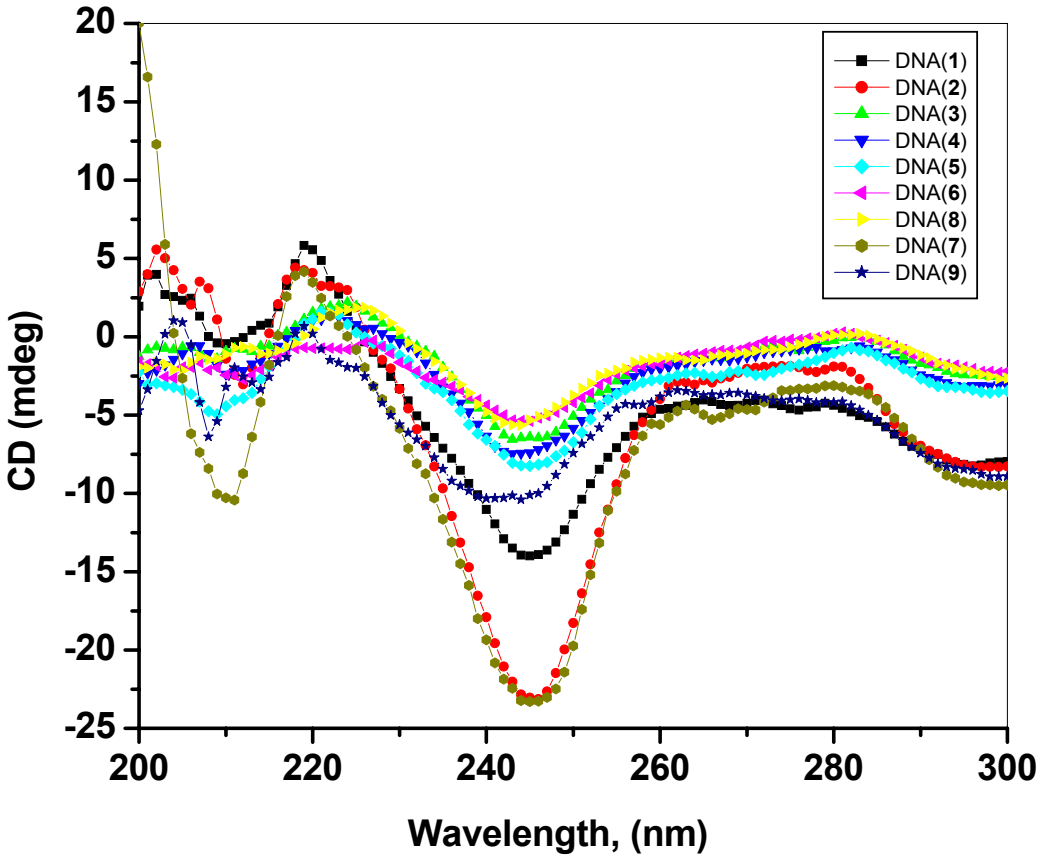

Figure S3: CD spectra profiles of the DNA-oligomers studied.

Table S1: Melting Temperature data for the DNA sequences studied.

DNA Sequence	**Tm (^{o}C)**
DNA(**1**)	50.1
DNA(**2**)	53.2
DNA(**3**)	53.8
DNA(**4**)	53.7
DNA(**5**)	54.0
DNA(**6**)	64.1
DNA(**7**)	60.3
DNA(**8**)	53.4
DNA(**9**)	53.0

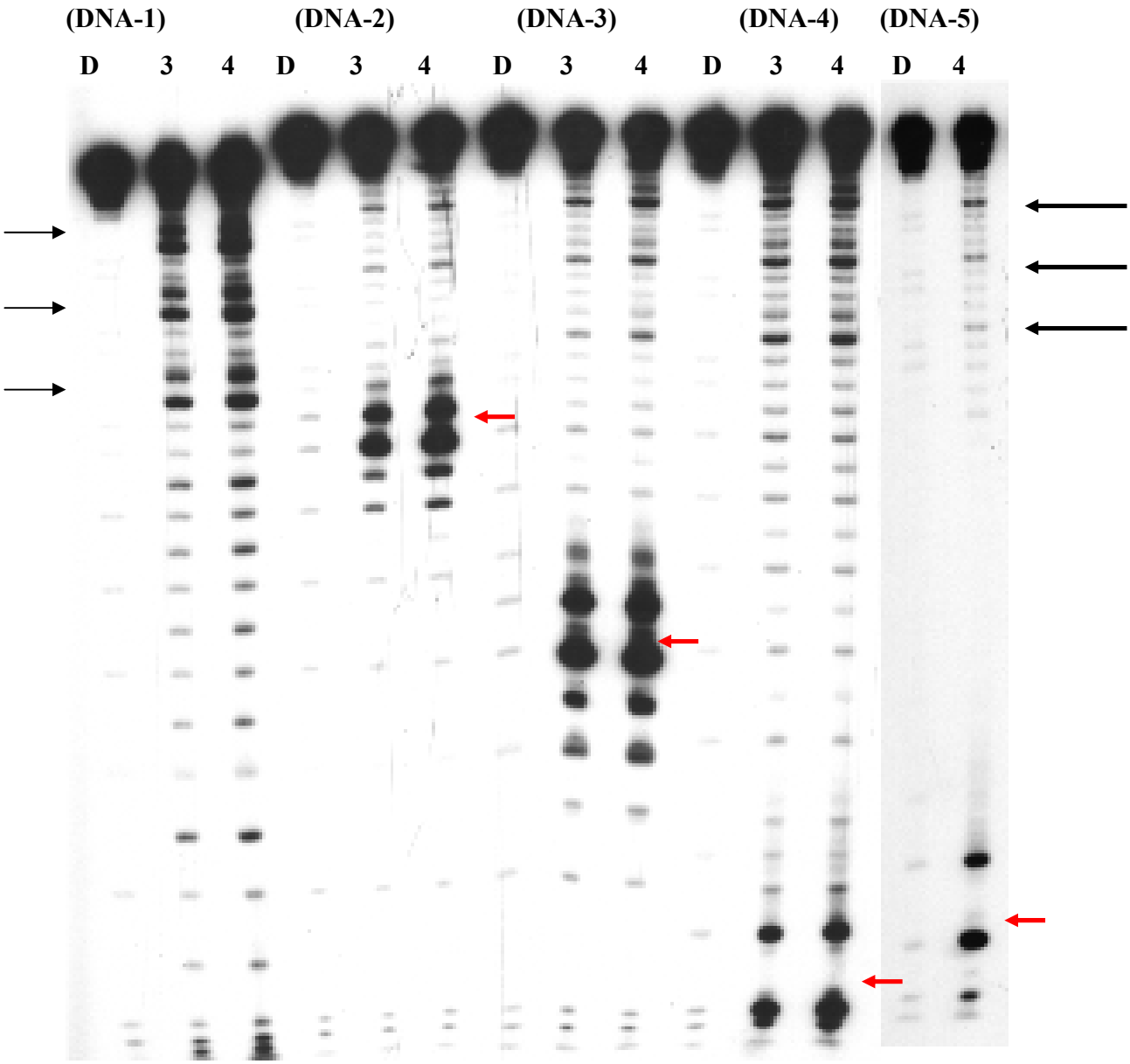

Figure S4: Autoradiogram showing the results of irradiation of DNA (**1**)- DNA(**5**). The TT steps (black) and GG steps (red) are indicated by arrows. The lanes correspond to minutes of irradiation (D, dark control or zero minutes; 3 is three minutes; 4 is for four minutes).

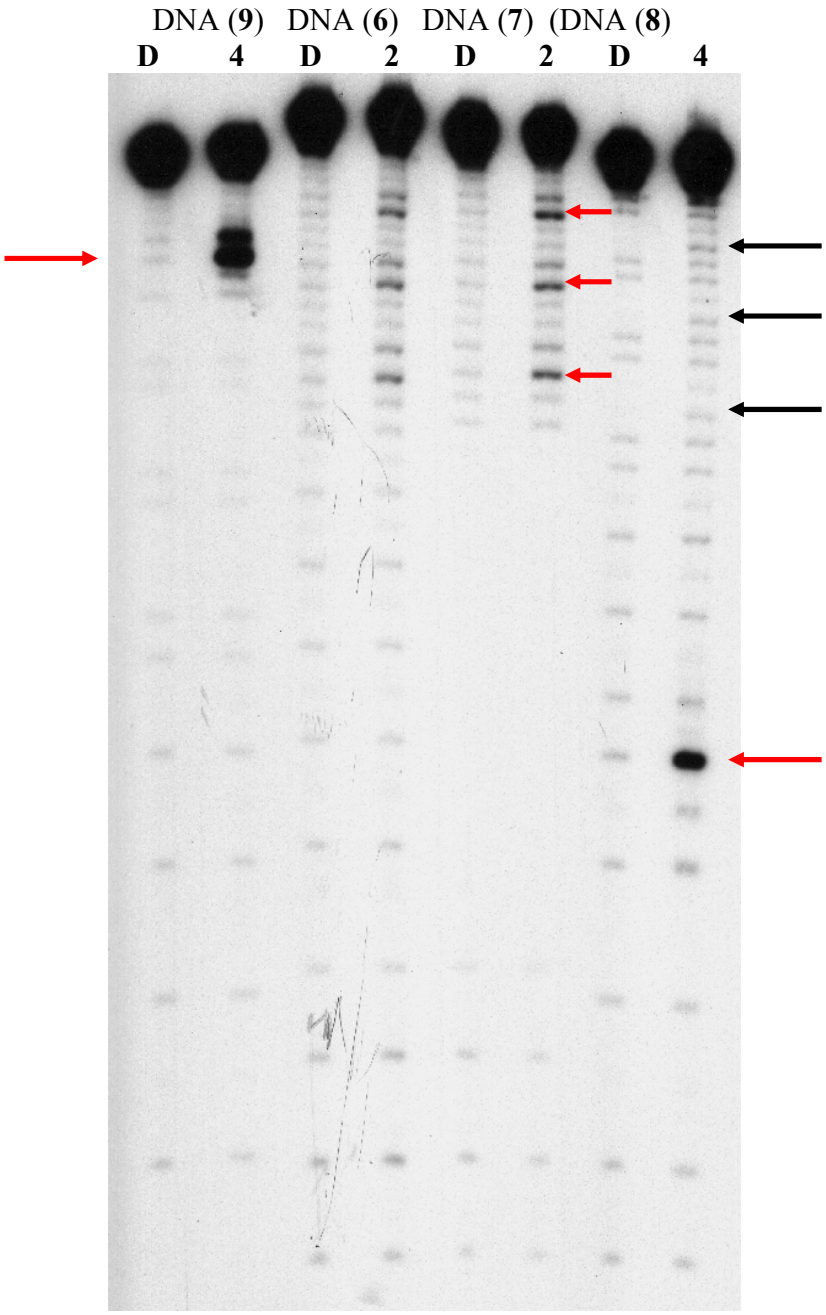

Figure S5: Autoradiogram showing the results of irradiation of DNA (**6**)- DNA(**9**). The TT steps (black) and GG steps (red) are indicated by arrows. The lanes correspond to minutes of irradiation (D, dark control or 0 minutes; 4 is 4 minutes; 2 is 2 minutes)