Supporting Information

Noncovalent DNA Binding Drives DNA Alkylation by Leinamycin. Evidence That the *Z*,*E*-5-(Thiazol-4-yl)-penta-2,4-dienone Moiety of the Natural Product Serves As An Atypical DNA Intercalator

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Materials. Reagents were purchased from the following suppliers: 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), *N,N,N',N'*-tetraethylenediamine (TEMED), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Company; dimethyl sulfate and sodium acetate were purchased from Aldrich Chemical; G-25 Sephadex, daunomycin, 4-nitrobenzenethiol, glutathione, 4-methylbenzenethiol, and 2-mercaptoethanol were purchased from Sigma-Aldrich; diethylenetriaminepentaacetic acid (DETAPAC) was purchased from Fluka Chemical Company; piperidine was purchased from Alfa Aesar; xylene cyanol, bromophenol blue, formamide, urea and ammonium persulfate were purchased from United States Biochemical; herring sperm DNA and TRIS-borate-EDTA (TBE) were purchased from Roche Molecular Biochemicals; acrylamide was purchased through Fischer Scientific Inc., T4 polynucleotide kinase (T4-PNK) and bovine serum albumin (BSA) were purchased from New England Biolabs; [γ-^{32}P]-dATP was purchased from Perkin-Elmer Life Sciences, and oligonucleotides were purchased from Integrated DNA Technologies. Leinamycin was generously provided by researchers at Kyowa Hakko Kogyo, Ltd. All solvents used were HPLC grade or higher and were purchased through Fischer Scientific, Inc.

Alkylation of Single-Stranded and Double-Stranded DNA by Activated Leinamycin. The single-stranded 2'-deoxyoligonucleotide 5'-TATTTATAACGCATTTAATTT-3' was labeled using [γ-^{32}P]dATP and T4 polynucleotide kinase according to standard protocols[1] and was purified on a 20% denaturing polyacrylamide gel.[1] The purified single-strand radiolabeled oligonucleotide was annealed with 1.5 equiv of the

complementary strand 5'-AAATTAAATGCGTTATAAATA-3' by warming to 95 ˚C for 5 min in buffer (25 mM MOPS, pH 7, and 100 mM NaCl), followed by slow-cooling to room temperature overnight. In a typical alkylation reaction, 5'-^{32}P labeled oligonucleotide (3 μL of single-stranded DNA or duplex DNA, approximately 50,000 cpm), HEPES buffer (2 μL of a 500 mM aqueous solution, pH 7), DETAPAC (0.5 μL of a 40 mM aqueous solution), 2-mercaptoethanol (1 μL, 2.5 μL, or 5 μL of a 4 mM aqueous solution or 2.5 μL of a 40 mM aqueous solution), and leinamycin (0.3 μL, 0.7 μL, 1.5 μL or 7.4 μL of a 13.5 mM solution in acetonitrile) were mixed with an appropriate amount of distilled, deionized water to give a final volume of 20 μL and the solution agitated using a vortex mixer and incubated at 37 ˚C for 23.3 h (final concentrations: 50 mM HEPES, pH 7; 1 mM DETAPAC; labeled DNA, 2'-deoxyoligonucleotide approximately 50,000 cpm; 0.2 mM, 0.5 mM, 1 mM or 5 mM of 2-mercaptoethanol; 0.2 mM, 0.5 mM, 1 mM or 5 mM of leinamycin; 37% (v/v) acetonitrile; in a final reaction volume of 20 μL). The reactions were stopped by precipitation of the DNA. Herring sperm carrier DNA (3 μL of a 1 mg/mL solution), sodium acetate (2 μL of a 3 M solution, pH 5), and absolute ethanol (175 μL) were added to the reaction mixture (final concentrations: 3 μg of carrier DNA, 30 mM of sodium acetate, 87.5% ethanol, in a final volume 200 μL). The mixture was cooled on dry ice for 45 min, followed by centrifuging for 45 min at 14,000 rpm in a benchtop microcentrifuge. The supernatant was removed, and the pellet washed twice with 80% ethanol−water (v/v). The DNA pellet was redissolved in aqueous piperidine (100 μL of a 0.5 M solution) and incubated at 90 ˚C for 25 min.[2] The solution was frozen on dry ice, lyophilized for 1.5 h in a SpeedVac Concentrator at 37 ˚C. The oligonucleotides were then taken through two

cycles of redissolution in 90 μL of water, frozen, and lyophilization at 37 ˚C. The dried DNA fragments were dissolved in formamide loading buffer, heated at 90 ˚C for 4 min and immersed in ice water. An equal number of counts were loaded in each lane of a 20% denaturing polyacrylamide gel (19:1 cross-linking, containing 5 M urea). The gel was electrophoresed at 1000 V for approximately 3 h. The DNA fragments in the gel were visualized and quantitated by phosphorImager analysis (Molecular Imager® FX, Imaging Screen-K, cat 170-7841, Bio-Rad, using Quantity One® Version 4.2.3, Bio-Rad).

Alkylation of Duplex DNA by Activated Leinamycin in the Presence of Varying Concentrations of Daunomycin. The single-stranded 2'-deoxyoligonucleotide 5'-GTT CGT ATA T**GG GAG G**TC **G**CA TGT **G**-3' was labeled and annealed with its corresponding complementary strand as described above (the underlined portion of the sequence is double-stranded). In a typical alkylation reaction, 5'-^{32}P-labeled oligonucleotide duplex (3 μL, approximately 50,000 cpm), TNE buffer (2 μL of a 10x aqueous solution, pH 7), daunomycin (0.2 μL, 1 μL, 2 μL, 4 μL, or 8 μL of a 10 μM aqueous solution and 0.24 μL, 0.32 μL, 0.4 μL, 0.8 μL, 1.2 μL, 1.6 μL, or 2 μL of a 0.5 mM aqueous solution), 2-mercaptoethanol (2.5 μL of a 4 mM aqueous solution), and leinamycin (1 μL of a 1 mM solution in acetonitrile) were mixed with an appropriate amount of distilled, deionized water and acetonitrile to give a final volume of 20 μL and the solution agitated using a vortex mixer (final concentrations: 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA), pH 7; 0.1 μM, 0.5 μM, 1 μM, 2 μM, 4 μM, 6 μM, 8 μM, 10 μM, 20 μM, 30 μM, 40 μM, or 50 μM daunomycin; 0.5 mM of 2-mercaptoethanol; 50 μM of leinamycin; 5% (v/v) acetonitrile). The reactions were incubated at 25 ˚C for 23.25 h,

followed by ethanol precipitation as described above. After piperidine workup, the DNA fragments were resolved on a 20% polyacrylamide sequencing gel and electrophoresed at 800V for approximately 9 h. The total yield of cleavage at the guanine residues was quantitatively compared to the remaining uncut DNA by phosphorimager analysis to yield the percent cleavage in each reaction.

Unwinding of Supercoiled Plasmid DNA by Activated Leinamycin. In a typical reaction, sodium phosphate buffer (2.5 μL of a 500 mM aqueous solution, pH 7), PGL2BASIC plasmid-DNA (3 μL of a 1 μg/mL solution aqueous solution), 2-mercaptoethanol (2.5 μL of a 20 mM aqueous solution), and leinamycin (1 μL or 2 μL of a 0.5 mM solution in acetonitrile, 2 μL or 2.5 μL of a 1 mM solution in acetonitrile, 0.74 μL, 1 μL, 1.5 μL, 2 μL, or 2.5 μL of a 5 mM solution in acetonitrile, 1.5 μL, 1.75 μL, 2 μL, 2.25 μL, or 2.5 μL of a 10 mM solution in acetonitrile) were mixed with an appropriate amount of distilled, deionized water and acetonitrile to give a final volume of 25 μL and the solution agitated using a vortex mixer (final concentrations: 50 mM sodium phosphate buffer, pH 7; 3 μg of PGL2BASIC plasmid-DNA; 2 mM of 2-mercaptoethanol; 0.02 mM, 0.04 mM, 0.08 mM, 0.1 mM, 0.15 mM, 0.2 mM, 0.3 mM, 0.4 mM, 0.5 mM, 0.6 mM, 0.7 mM, 0.8 mM, 0.9 mM, or 1.0 mM of leinamycin; 24% (v/v) acetonitrile). Samples were gently vortexed, incubated for 50 min at 4 ˚C in a cold room, and mixed with glycerol loading buffer (7 µL) containing 0.25% bromophenol blue and 40% sucrose. Samples were loaded on a 2% agarose gel and electrophoresed at 40 V for approximately 16 h in a 4 ˚C cold room.

Treatment With Activated Leinamycin Causes Time- and Concentration-Dependent Increases in the Viscosity of DNA-Containing Solutions. These studies were carried out in a custom-made Ostwald-type flow viscometer (ACE Glass) with a 1 mL-volume sample size and a 100 s flow-time for distilled water at room temperature. The reactions and viscosity studies were performed at 4 ˚C in a cold-room. In a typical reaction, sodium phosphate buffer (100 μL of a 100 mM aqueous solution, pH 7), calf thymus DNA (285 μL of a 3.5 mM aqueous solution), 2-mercaptoethanol (100 μL of a 7.2 mM aqueous solution), and leinamycin (24 μL, 48 μL, 100 μL of a 5 mM solution in acetonitrile) were mixed with an appropriate amount of distilled, deionized water and acetonitrile to give a final volume of 1 mL and the solution agitated using a vortex mixer (final concentrations: 10 mM sodium phosphate buffer, pH 7; 1 mM calf thymus DNA; 0.72 mM of 2-mercaptoethanol; 0.12 mM, 0.24 mM, 0.5 mM of leinamycin; 10% (v/v) acetonitrile; 1 mL final reaction volume). Samples were placed in the flow-viscometer using a glass Pasteur pipette and mixed by gentle bubbling. The flow times were recorded and the relative viscosity of the solution at a given time-point was calculated as $(t-t_0)/t_0$ where t is the flow time of the actual data point, t_0 is the flow time of the solution without leinamycin.

Alkylation of Palindromic DNA Duplexes Containing 5'-GC and 5'-CG Sites by Activated Leinamycin. The palindromic 2'-deoxyoligonucleotide duplexes **9** and **10** were labeled and annealed as described above. In a typical alkylation reaction, 5'-^{32}P labeled oligonucleotide duplex (3 μL, approximately 50,000 cpm), HEPES buffer (2 μL of a 500 mM aqueous solution, pH 7), DETAPAC (2.5 μL of a 40 mM aqueous solution),

2-mercaptoethanol (0.25 μL of a 0.4 mM aqueous solution, 0.25 μL or 2.5 μL of a 4 mM aqueous solution, and 2.5 μL of a 40 mM aqueous solution), and leinamycin (0.2 μL, or 2 μL of a 1 mM solution in acetonitrile, 4 μL of a 5 mM solution in acetonitrile, and 4.7 μL of a 42.6 mM solution in acetonitrile) were mixed with an appropriate amount of distilled, deionized water and acetonitrile to give a final volume of 20 μL and the solution agitated using a vortex mixer. Substoichiometric amounts of thiol were employed to avoid any potential scavenging of activated leinamycin by the thiol activating agent. The reactions were incubated at 30 ˚C for 22.75 h, followed by ethanol precipitation as described above. After piperidine work up, the resulting DNA fragments were loaded on a 20% polyacrylamide sequencing gel and electrophoresed at 1000V for approximately 3 h. The yield of cleavage at the guanine residue was quantitatively determined by phosphorimager analysis.

Alkylation of Bulge-Containing DNA Duplexes by Activated Leinamycin. The 2'-deoxyoligonucleotide duplexes **11-15** were labeled and annealed as described above. In a typical alkylation reaction, 5'-^{32}P labeled oligonucleotide duplexes were reacted with leinamycin (20 μM) in the presence of 2-mercaptoethanol (200 μM) and MOPS buffer (50 mM, pH 7.0), Herring Sperm DNA (200 μM) and sodium chloride (100 mM) for 2 h. Samples were extracted with an equal volume of butanol (2x). DNA was ethanol precipitated and the pellets were washed with 80% cold ethanol. The DNA pellets were redissolved in piperidine (200 mM aqueous solution) and incubated at 95 °C for 30 min. After cooling, samples were loaded onto a denaturing polyacrylamide gel (16%) and

electrophoresed for 3 h at 1200 V in TAE buffer. The labeled DNA fragments in the gel were visualized by phosphoimager analysis.

Alkylation of 5-Methylcytosine-Containing Duplexes by Activated Leinamycin. The 2'-deoxyoligonucleotide duplexes **16-19** were labeled and annealed as described above. In a typical alkylation reaction, 5'-^{32}P labeled oligonucleotide duplex (3 μL, approximately 50,000 cpm), HEPES buffer (2 μL of a 500 mM aqueous solution, pH 7), DETAPAC (2.5 μL of a 40 mM aqueous solution), 2-mercaptoethanol (5 μL of a 4 mM aqueous solution), and leinamycin (1.5 μL of a 13.5 mM solution in acetonitrile) were mixed with an appropriate amount of distilled, deionized water to give a final volume of 20 μL and the solution agitated using a vortex mixer (final concentrations: 50 mM HEPES, pH 7; 5 mM DETAPAC; labeled DNA, approximately 50,000 cpm; 1 mM of 2-mercaptoethanol; 1 mM of leinamycin; 7.5% (v/v) acetonitrile; 20 μL final reaction volume). The reactions were incubated at 37 ˚C for 23.3 h, followed by ethanol precipitation as described above. After piperidine work up, DNA fragments were resolved by electrophoresis on a 20% polyacrylamide sequencing gel. The gel was electrophoresed at 1000 V for approximately 2.5 h. The yield of cleavage at the target guanine residue on the labeled strand was quantitatively determined by phosphorimager analysis.

Literature Cited

(1) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Lab Manual*; Cold Spring Harbor Press: Cold Spring Harbor, NY, 1989.

(2) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499.