Benzo[c]phenanthrene Adducts and Nogalamycin Inhibit DNA Transesterification by Vaccinia Topoisomerase*\textsuperscript{S}

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Vaccinia DNA topoisomerase forms a covalent DNA-(3′-phosphotyrosyl)-enzyme intermediate at a specific target site 5′-CCCTT \textsuperscript{2}A and 5′-\textsuperscript{2}T\textsuperscript{1}p | N~−1 in duplex DNA. Here we study the effects of position-specific DNA intercalators on the rate and extent of single-turnover DNA transesterification. Chiral C-1 \& R S trans-opened 3,4-diol 1,2-epoxide adducts of benzo[c]phenanthrene (BcPh) were introduced at single N²-deoxyguanosine and N²-deoxyadenosine positions within the 3′-G³G⁺G³A²A²1′T¹A⁻² sequence of the nonscissile DNA strand. Transesterification was unaffected by BcPh intercalation between the +6 and +5 base pairs, slowed 4-fold by intercalation between the +5 and +4 base pairs, and virtually abolished by BcPh intercalation between the +4 and +3 base pairs and the +3 and +2 base pairs. Intercalation between the +2 and +1 base pairs by the +2R BcPh da adduct ablated transesterification, whereas the overlapping +1S BcPh da adduct slowed the rate of transesterification by a factor of 2700, with little effect upon the extent of the reaction. Intercalation at the scissile phosphodiester (between the +1 and −1 base pairs) slowed transesterification by a factor of 450. BcPh intercalation between the −1 and −2 base pairs slowed cleavage by two orders of magnitude, but intercalation between the −2 and −3 base pairs had little effect. The anthracycline drug nogalamycin, a non-covalent intercalator with preference for 5′-TG dimer adducts, inhibited the single-turnover DNA cleavage reaction of vaccinia topoisomerase with an IC\textsubscript{50} of 0.7 \textmu M. Nogalamycin was most effective when the drug was pre-incubated with DNA and when the cleavage target site was 5′-CCCTT | G instead of 5′-CCCTT | A. These findings demarcate upstream and downstream boundaries of the functional interface of vaccinia topoisomerase with its DNA target site.

Poxvirus DNA topoisomerase I is important for virus replication (1) and a potential target for drug therapy of smallpox, in light of its unique DNA recognition specificity, compact structure, and distinctive pharmacological sensitivities compared with human topoisomerase I (2–6). Poxvirus topoisomerases are exemplary type IB family members; they cleave and

\textsuperscript{*} Supported by National Institutes of Health Grants AI053471 and GM46330 (to S. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\textsuperscript{\S} The on-line version of this article (available at http://www.jbc.org) contains Table S1.

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exclusion of essential constituents of the topoisomerase from the DNA minor groove.

PAH diol epoxide-DNA adducts have also been exploited to probe the effects of position-specific intercalation, by introducing trans-opened 7,8-diol 9,10-epoxide adducts of BP at the exocyclic N\(^{3}\)-amino group of deoxyadenosine (dA) positions within the nonscissile strand (26). The R and S BP dA adducts intercalate from the major groove on the 5' and 3' sides of the modified base, respectively, and perturb local base stacking (29–33). R and S BP dA modifications at +1A reduced the transesterification rate by a factor of 700 to 1000 without affecting the yield of the covalent topoisomerase-DNA complex. BP dA modifications at +2A reduced the extent of transesterification and elicited rate decrements of 200-fold and 7000-fold for the S and R diastereomers, respectively (26). In contrast, BP dA adducts at the −2 position had no effect on the extent of the reaction and relatively little impact on the rate of cleavage. The BP dA interference effects demarcated the −1 base pair as the “downstream” margin of the functional interface between DNA and vaccinia topoisomerase that can be affected significantly by BP intercalation. The “upstream” margin remained undefined because the effects of intercalating PAH adducts at the guanine positions of the cleavage target site were not tested.

Here we extend the use of defined PAH diol epoxide-DNA adducts to probe the effects of position-specific intercalation by benzo[c]phenanthrene (BcPh) at all purines of the topoisomerase target sequence. BcPh exemplifies the sterically hindered, nonplanar “fjord-region” class of PAHs. BcPh has been studied extensively in light of the highly tumorigenic and mutagenic properties of metabolically activated BcPh diol epoxides, which react at the benzylic C1 position by trans addition of guanine N\(^{2}\) and adenine N\(^{6}\) in DNA to form the covalent trans 1S and 1R BcPh dG and BcPh dA adducts depicted in Fig. 1. Structures of duplex DNAs containing single BcPh dG adducts have established that the aromatic ring systems intercalate from the minor groove on opposite sides of the modified dG base depending on their stereochemical configuration (34). The S diastereomer intercalates on the 5’ side of the dG, whereas the R diastereomer intercalates on the 3’ side (Fig. 2). The intercalated BcPh dG and BcPh dA adducts do not disrupt base pairing, but they do cause a buckling of the modified base pair and the unmodified base pair flanking the hydrocarbon (Fig. 2). An attractive feature of BcPh adducts with respect to studies of vaccinia topoisomerase is that they provide structural probes for intercalative interference effects at all of the purine bases of the nonscissile strand of the 5'-CCCTT/3'-GGGAA target site and for effects of intercalation at positions immediately 3' of the scissile phosphodiester.

We report here that transesterification by vaccinia topoisomerase was suppressed by BcPh intercalation between the +4/+3, +3/+2, +2/+1, +1/-1, and −1/−2 base pairs, but intercalation between the +6/+5, +5/+4, and −2/−3 base pairs had little effect. These new findings demarcate upstream and downstream margins of the interface between DNA and vaccinia topoisomerase.

In light of these results, we tested the effects of the anthracycline drug nogalamycin, which intercalates preferentially at 5'-TG dinucleotides in duplex DNA (37–39). We find that nogalamycin inhibits the single-turnover DNA cleavage reaction of vaccinia topoisomerase and that its potency is higher when the cleavage target site is 5'-CCCTT ↓ G versus 5'-CCCTT ↓ A. We surmise that nogalamycin can inhibit the vaccinia enzyme by intercalating between the +1 and −1 base pairs.
Escherichia coli produced in depicted in the figures. annealed duplexes and the sequences of the component strands are mixed phosphoramidites as described (40). Oligonucleotides containing BcPh dG/H11032, O\text{adducts (Fig. 1) were synthesized from appropriately protected 5'-cyanepoxide adducts are illustrated looking into the minor groove. The ° nucleotides as specified were heated to 80°C and diluted freshly with 0.1% SDS. Free DNA migrated near the dye front. Covalent ionization-mass spectrometry analysis. The oligonucleotides were confirmed by matrix-assisted laser desorption ionization-mass spectrometry analysis.

**DNA Cleavage Substrates**—The CCCTT-containing scissile strands were 5'-32P-labeled by enzymatic phosphorylation in the presence of [γ-32P]ATP and T4 polynucleotide kinase. The labeled oligonucleotides were gel-purified and hybridized to standard or modified nonscissile strand oligonucleotides at a 1:4 molar ratio of scissile:nonscissile strand. Annealing reaction mixtures containing 0.2 M NaCl and oligonucleotides as specified were heated to 80 °C and then slow-cooled to 22 °C. The hybridized DNAs were stored at 4 °C. The structures of the annealed duplexes and the sequences of the component strands are depicted in the figures.

**Vaccinia Topoisomerase**—Recombinant vaccinia topoisomerase was produced in *Escherichia coli* BL21 by infection with bacteriophage λC66 (2) and then purified to apparent homogeneity from the soluble bacterial lysate by phosphocellulose and Source S-15 chromatography steps. Protein concentration was determined by using the dye-binding method (Bio-Rad) with bovine serum albumin as the standard.

**Drugs**—Nogalamycin was obtained from Amersham Biosciences, courtesy of Dr. Paul Aristoff. The drugs were dissolved in Me2SO at a concentration of 10 mg/ml. Nogalamycin was obtained from Amersham Biosciences, courtesy of the Developmental Therapeutics Program, National Cancer Institute.

**RESULTS**

**Position-specific Drug Interference Effects on DNA Translesion Synthesis**—Oligodeoxynucleotide 18-mers containing a single C1 S or R BcPh diol epoxide adduct at purine positions of the nonscissile strand sequence 3'-G/T-G/T-G/T-A/V-A/T-A/- was synthesized and then annealed to a 5'-32P-labeled 34-mer scissile strand to form suicide cleavage substrates for vaccinia topoisomerase (Fig. 3). The cleavage translesion synthesis reaction results in covalent attachment of the 32P-labeled 12-mer -5'pCGTGTCGCCCTTptp to the enzyme via Tyr-274. The unlabeled 22-mer 5'-OH-leaving strand dissociates spontaneously from the protein-DNA complex. Loss of the leaving strand drives the reaction toward the covalent state, so that the reaction can be treated kinetically as a first-order unidirectional process.

The reaction of excess topoisomerase with the unmodified control substrate attained an end point at which 90% of the DNA was converted to covalent topoisomerase-DNA complex; the reaction was complete within 20 s. The extent of translesion synthesis after 5 s was 55% of the end point value. From this datum, we calculated a single-turnover cleavage rate constant (*k*<sub>c</sub>) of 0.27 s<sup>-1</sup> (Fig. 3).

We found that the S and R BcPh modifications at position +5G and the R BcPh modification at +4G slowed the translesion synthesis rate to 0.13, 0.07, and 0.07 s<sup>-1</sup>, respectively, without significantly affecting the yield of covalently bound DNA at the reaction end point (61–65%). The *k*<sub>c</sub> for cleavage of these BcPh dG-modified substrates did not increase when the concentration of topoisomerase in the reaction mixture was varied over a 4-fold range (not shown), indicating that the modestly slowed cleavage rates (2- to 4-fold compared with the unmodified control DNA) were not caused by slow noncovalent binding of topoisomerase to these substrates. In contrast, the S BcPh adduct at +4G, and the R and S BcPh adducts at +3G, and the S and R adducts at +2A virtually abolished the translesion synthesis reaction. The extents of DNA cleavage after a 24-h reaction in enzyme excess were in the range of 1–4% of the input suicide substrate (Fig. 3). Although the accumulation of the topoisomerase-DNA complex on these modified DNAs was time-dependent, the reaction clearly did not attain a useful end point for the purpose of calculating a cleavage rate constant. The end point did not increase when the concentration of topoisomerase was doubled, implying that the reaction was not limited by the noncovalent binding step.
Effects of BcPh diol epoxide adducts on the rate and extent of single-turnover transesterification by vaccinia topoisomerase. The 34-mer/18-mer suicide substrate is shown at the bottom of the figure, with the site of cleavage indicated by a vertical arrow. The 5′-32P-label on the scissile strand is denoted by an asterisk. The unmodified control substrate is depicted in greater detail, with the phosphodiester backbone drawn as horizontal lines and the base pairs drawn as vertical lines. The numerical coordinates of the nucleotides are indicated above the control sequence. The S and R diastereomers of the BcPh adducts are depicted as intercalated vertical bars in their respective orientations on the 3′ side or 5′ side of the site of covalent attachment to purine bases on the non-scissile strand. Cleavage rate constants and reaction end points for transesterification by vaccinia topoisomerase are indicated to the right of each structure.

FIG. 3. Effects of BcPh diol epoxide adducts on the rate and extent of single-turnover transesterification by vaccinia topoisomerase. The 34-mer/18-mer suicide substrate is shown at the bottom of the figure, with the site of cleavage indicated by a vertical arrow. The 5′-32P-label on the scissile strand is denoted by an asterisk. The unmodified control substrate is depicted in greater detail, with the phosphodiester backbone drawn as horizontal lines and the base pairs drawn as vertical lines. The numerical coordinates of the nucleotides are indicated above the control sequence. The S and R diastereomers of the BcPh adducts are depicted as intercalated vertical bars in their respective orientations on the 3′ side or 5′ side of the site of covalent attachment to purine bases on the non-scissile strand. Cleavage rate constants and reaction end points for transesterification by vaccinia topoisomerase are indicated to the right of each structure.

Rather, we surmise that the majority of the topoisomerase binding events on the +4S, +3R, and +3S BcPh dG and +2S and +2R BcPh dA substrates were nonproductive with respect to transesterification, and that there was not a free equilibrium between productive and nonproductive binding modes (at least not within the 24-h period that the reactions were monitored).

The S and R BcPh dA modifications at +1A reduced kcl to 0.0001 and 0.0006 s⁻¹, respectively, without significantly affecting the end point (67–71% cleavage). Thus, +1 BcPh dA adducts elicited rate decrements of 2700-fold and 450-fold for the S and R diastereomers, respectively. Note that the interference effect was greater when the +1 BcPh dA adduct was intercalated on the 3′ side of the modified adenine base facing away from the scissile phosphodiester (Fig. 3). The BcPh dA adducts at −2 position also displayed a strong orientation bias with respect to rate effects. The S diastereomer, which faces toward the scissile phosphodiester, reduced the cleavage rate constant to 0.003 s⁻¹, a 90-fold decrement compared with the unmodified control DNA, whereas the R diastereomer at −2 had little impact on the rate (0.12 s⁻¹). Neither of the −2 BcPh dA adducts affected the cleavage reaction end point.

To address whether BcPh substitutions altered the site of cleavage within the 34-mer scissile strand, the products of the cleavage reactions with unmodified DNA, and the +5R, +5S, +4R, +1R, −2S, and −2R BcPh diol epoxide-modified DNAs were digested with proteinase K in the presence of SDS to remove the covalently linked topoisomerase, and the radiolabeled DNA reaction products were then analyzed by denaturing polyacrylamide gel electrophoresis. Reaction of topoisomerase with the unmodified control substrate results in the appearance of a cluster of radiolabeled species migrating faster than the input 32P-labeled 34-mer strand, which consists of the 12-mer 5′-pCGTGTCGCCCTTp linked to one or more amino acids of the topoisomerase. The same cluster was produced by proteinase K digestion of the covalent complex formed by reaction of topoisomerase with the BcPh dA and BcPh dG substrates (data not shown). Thus, the site of covalent complex formation was unchanged by the BcPh modifications. Any shift in the cleavage site, and hence the size of the covalently bound oligonucleotide, would have been readily detected by an altered mobility of the array of labeled oligonucleotide-peptide complexes.

Effects of Nogalamycin on DNA Transesterification by Vaccinia Topoisomerase—The exquisite sensitivity of vaccinia topoisomerase to position-specific intercalators has implications for the discovery of poxvirus-specific topoisomerase inhibitors and/or poisons as candidate antiviral drugs. DNA intercalating agents have been widely studied as inhibitors of other DNA topoisomerases; indeed, intercalating drugs that inhibit DNA topoisomerase II are mainstays of cancer chemotherapy. Existing compounds that display some activity against microbial or eukaryotic cellular topoisomerases are a reason-
Here we tested the effects of nogalamycin on the rate and extent of single-turnover cleavage by vaccinia topoisomerase (Fig. 5). In these experiments, the suicide substrates were made by annealing a 5'-32P-labeled 18-mer scissile strand to an unlabeled 30-mer strand. We tested two different cleavage site sequences, 5'-CCCTTpG and 5'-CCCTTpA, that differed with respect to the −1 base pair immediately 3' of the scissile phosphodiester. The DNA was preincubated for 10 min with increasing concentrations of nogalamycin, and the DNA-drug mixtures were then reacted for 15 s with vaccinia topoisomerase, which was present in excess over the 32P-labeled DNA. The use of a short reaction time afforded reasonable sensitivity to drug effects on the rate of topoisomerase transesterification. In control reactions containing 10% Me2SO and no drug, 70–80% of the input DNA was converted to covalent topoisomerase-DNA complex in 15 s. Nogalamycin elicited a concentration-dependent inhibition of transesterification with both substrates. The instructive finding was that the potency of nogalamycin as a DNA cleavage inhibitor was acutely dependent upon the −1 base pair immediately 3' of the scissile phosphodiester. The DNA was preincubated for 10 min with nogalamycin, and the extent of covalent complex formation is plotted as a function of drug concentration. The order of addition of the reaction components also had a profound effect on nogalamycin inhibition of vaccinia topoisomerase (Fig. 6A). In one series of reactions, nogalamycin was preincubated with the 5'-CCCTTpG DNA substrate for 10 min prior to initiating the transesterification reaction by the addition of topoisomerase. In another reaction series, nogalamycin was preincubated with topoisomerase for 10 min prior to initiating the transesterification reaction by adding the DNA. In both series, the DNA cleavage reactions were quenched after 15 s. The striking result was that nogalamycin had to be exposed to the DNA substrate prior to adding topoisomerase to exert its inhibitory effects. For example, 10 μM nogalamycin reduced cleavage by 98% when added to DNA first, but only 19% when added to topoisomerase first (Fig. 6A). Taken together, the data in Figs. 5 and 6A suggest that nogalamycin exerts its effect on the cleavage reaction of vaccinia topoisomerase by virtue of binding to the DNA, not to the topoisomerase.

Nogamycin is an analog of nogalamycin that lacks the methyl ester on the A ring (Fig. 4). Nogamycin inhibited DNA cleavage by vaccinia topoisomerase in a concentration-dependent fashion when the drug was preincubated with the DNA substrate (IC50 1.5 μM and IC90 10 μM), but was less effective when the order of addition was reversed, so that nogamycin was preincubated with the topoisomerase and the reactions were initiated by adding DNA (Fig. 6B).

A kinetic analysis of single-turnover cleavage of DNA preincubated with 10 μM drug is shown in Fig. 6C. The apparent cleavage rate constants (and reaction endpoints) were 0.006 s⁻¹ (89% of input DNA cleaved) and 0.0005 s⁻¹ (77% of input DNA cleaved) for nogamycin and nogalamycin, respectively. It is noteworthy that the impact of nogalamycin on the rate of DNA cleavage is virtually identical to that elicited by intercalation of BcPh between the +1 and −1 bases flanking the scissile phosphodiester.

The topoisomerase catalytic cycle includes two transesterification reactions, cleavage and religation. Religation entails the attack of the DNA 5'-OH on the covalent intermediate, leading to expulsion of the Tyr-274 leaving group and restoration of the DNA phosphodiester backbone. The effect of nogalamycin on the religation reaction was studied under single-turnover conditions by assaying the ability of pre-formed suicide intermediate to transfer the covalently held 5'-32P-labeled 12-mer strand to a 5'-OH-terminated 18-mer strand to form a 30-mer product (5, 50). The reaction mixtures containing the suicide intermediate were adjusted to 10% Me2SO and 0, 5, 10, or 20 μM nogalamycin and incubated for 5 min at 37 °C. Religation was initiated by the simultaneous addition of NaCl to 0.5 M and a 50-fold molar excess of a 5'-hydroxyl-terminated 18-mer...
acceptor strand d(GTTCCGATAGTGACTACA) complementary to the 5' single-stranded tail of the suicide intermediate. The religation reaction was quenched after 15 s. The extent of religation (expressed as the percent of input 32P-labeled DNA converted to 30-mer) was 77, 76, 75, and 75% in reactions containing 0, 5, 10, and 20 μM nogalamycin, respectively (data not shown). These results suggest that nogalamycin interferes selectively with the forward cleavage reaction of vaccinia topoisomerase.

**DISCUSSION**

**Position-specific Intercalation Interference by Covalent BcPh Diol Epoxide Adducts**—By introducing R and S BcPh adducts at all purine positions of the nonacisle strand sequence 3'-GGGAATA complementary to the 5'-CCCTTAT target site, we have defined the upstream and downstream margins of the functional interface of vaccinia topoisomerase with DNA containing an intercalated PAH. We observe a discrete and abrupt upstream boundary for BcPh interference effects, whereby transesterification was unaffected by BcPh intercalation between the +6 and +5 base pairs, slowed modestly by intercalation between the +5 and +4 base pairs, and virtually abolished by BcPh intercalation between the +4 and +3 base pairs. It is noteworthy that identical 4-fold rate effects were seen for the +5S and +4R BcPh adducts, which intercalate between +5G and +4G from different orientations. Identical drastic inhibition of cleavage occurs when the +4S and +3R BcPh dG adducts intercalate from different directions between +4G and +3G bases.

The profound suppression of cleavage persists when BcPh insinuates between +3G and +2A, whether it intercalates from the upstream side via the minor groove (+3S BPhG) or from the downstream side via the major groove (+2S BcPh dA). It was striking that the +2R BcPh dA adduct suppressed the yield of the topoisomerase-DNA intermediate, whereas +1S BcPh dA adduct, which insinuates into the same dinucleotide step, affected only the rate of cleavage. We presume that subtle differences in space occupancy by the intercalated ring systems or their induced DNA distortions are responsible for the different effects of the +2R and +1S BcPh dA adducts on topoisomerase cleavage. This orientation disparity for the BcPh dA adducts is reminiscent of the orientation-biased effects of benz[a]pyrene intercalation into the same dinucleotide step.

Placement of the BcPh adduct progressively closer to, and then past, the cleavage site resulted in a gradual alleviation of the interference effects. BcPh intercalation at the scissile phosphodiester (between the +1 and −1 base pairs) slowed transesterification to 0.0006 s⁻¹. The identical rate cleavage constant was reported previously when benz[a]pyrene was intercalated into the same niche from the +1A base (26). BcPh intercalation between the −1 and −2 base pairs slowed cleavage by two orders of magnitude, but intercalation between the −2 and −3 base pairs had little effect. Thus the −2 base pair is the distal margin of the BcPh intercalation interference footprint.

The simplest interpretation of these data is that BcPh inhibition of transesterification is a consequence of disruption of contacts between the topoisomerase and the base pairs of the CCCTT target site that are critical to trigger DNA cleavage. This view is consistent with NMR data showing that single R and S BcPh adducts cause spreading and buckling of the base pairs immediately flanking the intercalated BcPh moiety (Fig. 2). Alternatively, the BcPh adducts may inhibit indirectly by perturbing the phosphodiester backbone.

A previous study by Pommier et al. (51) addressed the effects of S and R BcPh dG modifications of a scissile strand guanine base at a TpG cleavage site for human topoisomerase I (equivalent to the −1 base in our numbering system). They
found that the S BcPh dG diastereomer, which intercalates at the scissile phosphodiester (+1/−1), and the R diastereomer, which intercalates on the 3′ side of the guanine (between the −1 and −2 base pairs in our numbering), completely suppressed cleavage at the T ↓ G site and, instead, triggered cleavage at a new site two nucleotides upstream. The propensity of human topoisomerase I to switch cleavage sites when confronted with an unfavorable DNA lesion makes it difficult to obtain a quantitative picture of interference effects on transsterification by the human enzyme. Nonetheless, the suppressive effects of BcPh intercalation at +1/−1 and −1/−2 steps are qualitatively concordant for the human and vaccinia topoisomerases. As shown previously (25, 26), and confirmed here for BcPh, the cleavage site of vaccinia topoisomerase was unaltered by PAH modifications.

**Intercalation Interference by Nogalamycin**—Having demonstrated the potency of covalently fixed intercalators as inhibitors of DNA cleavage by vaccinia topoisomerase, we tested the effects of a well studied soluble intercalator, nogalamycin, which binds DNA in a sequence-dependent fashion. The inhibition of vaccinia topoisomerase by nogalamycin is concentration-dependent, requires preincubation of drug with the DNA, and is enhanced by the presence of a guanine at the N base flanking the CCCTT p ↓ N cleavage site. Given that TG is a preferred target site for nogalamycin intercalation, we posit that nogalamycin inhibits by intercalating between the +1 and −1 base pairs and thereby mimicking the action of the covalent 1R BcPh dA adduct, which elicits an effect similar to nogalamycin on the rate of single-turnover cleavage.

The order of addition effect on nogalamycin inhibition suggests that (i) the drug acts by binding to DNA rather than to topoisomerase, and (ii) the binding of topoisomerase to DNA is faster than the binding of nogalamycin to DNA. The latter point is consistent with experimental data (52, 53) and is sensible in light of the steric problems involved in inserting the bulky sugar appendages of the dumbbell-shaped nogalamycin molecule between the base pairs of duplex DNA. Once intercalated, nogalamycin dissociates very slowly from DNA (54). The kinetics of dissociation depend upon the DNA sequence complexity. Nogalamycin is released with a half-life of 50 min from the alternating copolymer poly(dG·dG)·poly(dA·dC), which consists exclusively of tandem TG dinucleotides that are preferred intercalation sites. Nogalamycin dissociates from calf thymus DNA with three apparent kinetic components, with half-lives of 5, 17, and 72 min, respectively (54).

Nogalamycin suppresses the forward cleavage transsterification reaction, but has little apparent effect upon religation by the suicide intermediate. Thus, nogalamycin can be classified as a poxvirus topoisomerase inhibitor rather than a poxvirus topoisomerase poison. The poisoning effect of nogalamycin on mammalian DNA topoisomerase I has been studied thoroughly by Liu and coworkers (48, 49). The covalent trapping of mammalian topoisomerase on DNA elicited by nogalamycin occurs only at a subset of topoisomerase cleavage sites. Detailed analysis of one such nogalamycin-stimulated cleavage site attributes the poisoning effect to the intercalation of nogalamycin at a TG step located six nucleotides upstream (5′) of the scissile phosphodiester (49). However, it has been shown that nogalamycin is also a concentration-dependent inhibitor of DNA cleavage by mammalian topoisomerase I at many other target sites in duplex DNA (48, 49). Thus, the outcome of nogalamycin intercalation with respect to the mammalian topoisomerase will depend upon the sequence context and the position of the intercalated drug relative to the cleavage site. From a pharmacological perspective, the topo I poison mechanism dominates cytotoxicity, which allows for drug efficacy even if the topoisomerase is nonessential for cell growth (55) and even if only a fraction of the potential topoisomerase-DNA complexes can be trapped by the drug.

In the case of vaccinia topoisomerase, we can reasonably surmise that nogalamycin is not acting as a poison on the model CCCTTTPG substrate employed herein for the analysis of the cleavage and religation reactions. We do not exclude the possibility that nogalamycin can have a modest slowing effect upon religation of this cleavage site, because the reaction is fast ($k_{rel} = 1 \times 10^7$) and the sensitivity of the 15-s single-turnover religation assay is such that a 10-fold rate decrement would not be detected. Also, we do not exclude the possibility that nogalamycin could poison vaccinia topoisomerase at a subset of target sites in viral DNA that have an especially suitable flanking sequence context. Because nogalamycin is not likely to intercalate at subtoxic concentrations within the conserved pentapyrimidine (T/C)CCCTT target site, and given that only a fraction of poxvirus topoisomerase cleavage sites in vivo will be flanked by a TpG dinucleotide, we suspect that the inhibitory mode of nogalamycin action will not provide for antiviral efficacy predicated on specific targeting of the poxvirus topoisomerase. Nonetheless, initial studies show that nogalamycin inhibits vaccinia virus replication in cell culture;2 it will be of interest to identify a molecular target for its antiviral action.

In conclusion, position-specific intercalation is a powerful means to block DNA cleavage by poxvirus topoisomerase and may, in principle, afford useful diffusible small-molecule inhibitors, provided that the compounds can be directed to the CCCTT target sequence, and small molecule poisons, provided that the compounds can be directed to the covalent topoisomerase-DNA intermediate.

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J. Biol. Chem. 2004, 279:23335-23342.
doi: 10.1074/jbc.M401203200 originally published online March 23, 2004

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