altered by further improvements in the computational methodology employed to calculate the singlet-triplet energy separation.

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Supplementary Material Available: Tables of UHF/ROHF/GVB/π-CI geometries of structures **1** and **2** (2 pages). Ordering information is given on any current masthead page.

Interaction of the Aryl Tetrasaccharide Domain of Calicheamicin γ_1^I with DNA: Influence on Aglycon and Methidiumpropyl-EDTA·Iron(II)-Mediated DNA Cleavage

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Abstract: This study assesses individual interactions of the oligosaccharide and aglycon domains of calicheamicin γ_1^I, an antitumor antibiotic, with DNA. Preincubation of DNA with the full carbohydrate domain followed by incubation with aglycon leads to sequence-selective cleavage not observed in the absence of the carbohydrate.

Calicheamicin γ_1^I (**1**) and esperamicin are DNA minor groove binding drugs bearing enediyne ("aglycon" sector) and carbohydrate moieties.[1,2] The drugs produce sequence-selective double-stranded cleavage at homopyrimidine/homopurine tracts on DNA duplexes,[3] via a 1,4-dehydrobenzene diradical produced in the aglycon sector upon bioreductive alkylation and cyclization.[2,4] Earlier studies demonstrated that the carbohydrate moieties of the drugs determine their sequence selectivity,[3b,5-8] possibly via an extended conformation spanning the minor groove.[5a]

In the research described herein we sought to learn whether the carbohydrate domain of the drug calicheamicin γ_1^I, independent of the aglycon, binds to a DNA duplex substrate (see Figure 1). The DNA sequence was selected on the basis of its demonstrated capacity to exhibit sequence-selective binding and cleavage by **1**.[7] The carbohydrate selected for this purpose was the methyl glycoside **2**, recently synthesized in two laboratories.[9] DNase I footprinting of **2** has provided evidence that the oligosaccharide does recognize drug-binding (**1**-binding) sites, but with perhaps lower specificity than the drug.[10] The availability through synthesis of the carbohydrate sector mimic **2**, as well as aglycon sector analog **5**,[11] allowed us to probe, more deeply, their individual interactions with the DNA substrate, thereby providing inferences as to their synergism in the intact drug.

We first examined the action of intact drug **1** on the DNA duplex which had been preincubated with oligosaccharide **2**. This experiment produced a dramatic decrease in drug-mediated cleavage (Figure 2, lanes 1-3). No such effect was observed when either sucrose (Figure 2, lanes 4 and 5) or even the azafuranose rearranged product of esperamicin trisaccharide[12] (**4**; Figure 2, lanes 6 and 7) was used. The trisaccharide domain mimic[13,14] (**3**) of esperamicin, a drug with markedly less sequence specificity than calicheamicin,[2c,d,15] inhibited drug-mediated (**1**-mediated) specific cleavage only to a very small extent, and at the highest concentration (1.5 mM) of oligosaccharide used.

Affinity of the oligosaccharide **2** for specific sequences on the DNA was determined by footprinting analyses of the top (hom-

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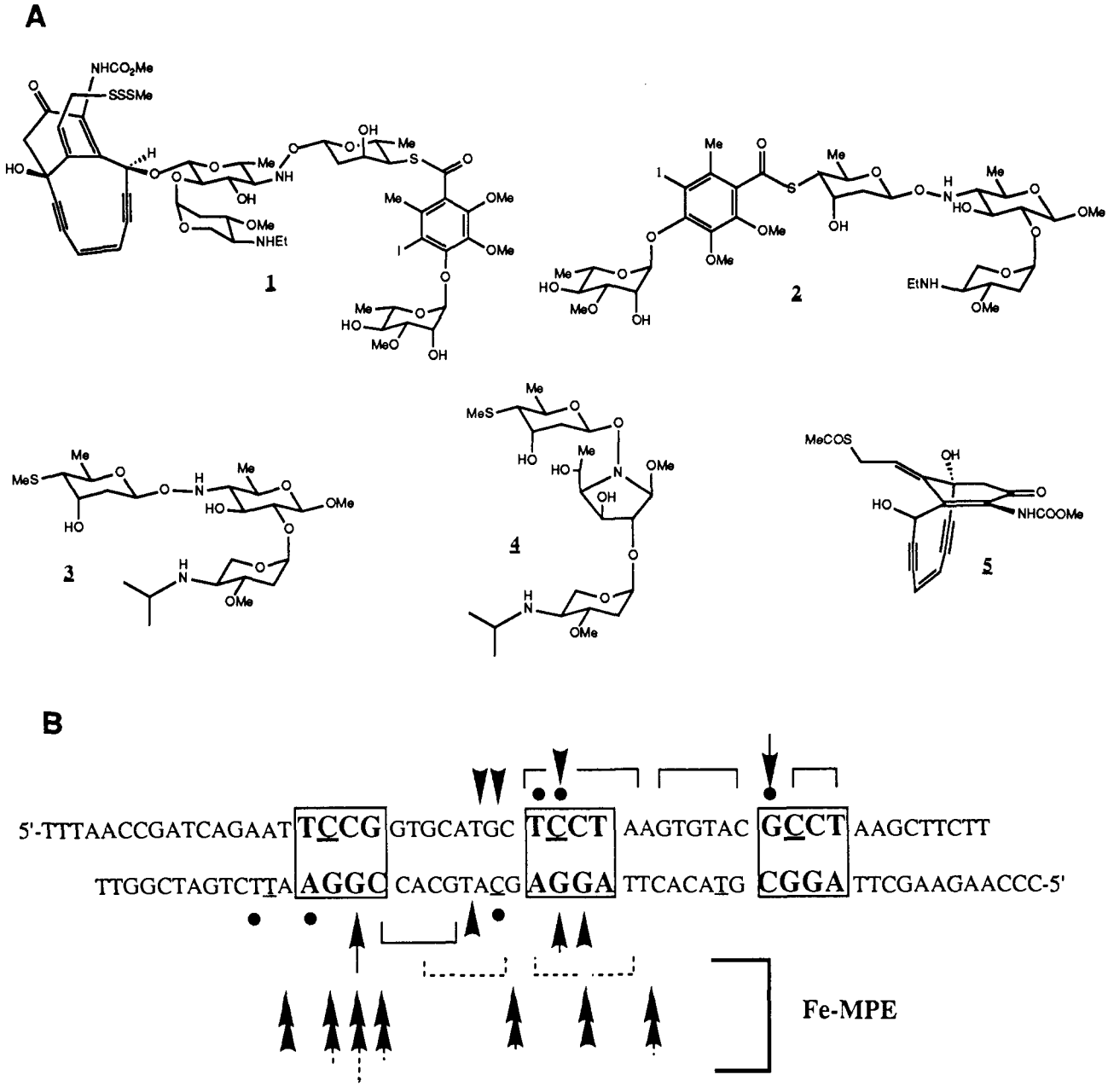

Figure 1. (A) Structures of calicheamicin γ_1^I (**1**), oligosaccharides **2–4**, and aglycon analog (**5**). (B) Sequence of the DNA duplex used in the sequence selectivity and relative binding affinity studies. Boxed elements are calicheamicin γ_1^I recognition sequences, with underlined bases marking expected cleavage sites[3a] and dotted bases showing experimentally observed drug cleavage sites. Arrows and double arrows mark strong aglycon and Fe-MPE cleavage sites, respectively, while solid and dotted brackets represent regions that are protected from aglycon and Fe-MPE cleavage, respectively.

opyrimidine) and bottom (homopurine) strands of the duplex. In the absence of carbohydrate, the calicheamicin γ_1^I aglycon analog **5** cleaved DNA in a nonspecific manner at every base, giving rise to a uniform ladder.[16] *At moderate oligosaccharide concentrations, a modulation of the uniformity was observed, with strong cleavage sites flanking bases which were relatively protected.* In the top strand, the strong cleavage sites coincided with some of the observed calicheamicin γ_1^I cleavage sites, whereas in the bottom strand, these sites were two to three nucleotides 5′ to observed calicheamicin cleavage sites and lay within the four-base calicheamicin recognition sequences. At very high oligosaccharide concentrations, an overall nonspecific inhibition of aglycon-mediated DNA cleavage was detected.[16]

Similar results were obtained with methidiumpropyl-EDTA·iron(II) (Fe-MPE), a nonspecific DNA-cleaving reagent.[17] As in the case of the aglycon, strong cleavage sites within the calicheamicin binding sites flanked regions which were protected.[16]

Densitometric analyses of the autoradiograms served to clearly visualize regions of protection both within and adjacent to calicheamicin binding sites (Figure 3A,B). The enhanced susceptibility to cleavage at certain bases (Figure 3A,B) is analogous to DNase I footprints of several antibiotics described in earlier studies[17b,c,18] and may be indicative of the bound oligosaccharide altering the width of the minor groove at the binding site and at flanking sequences.[18d] Chromomycin, a nonintercalative, G·C base

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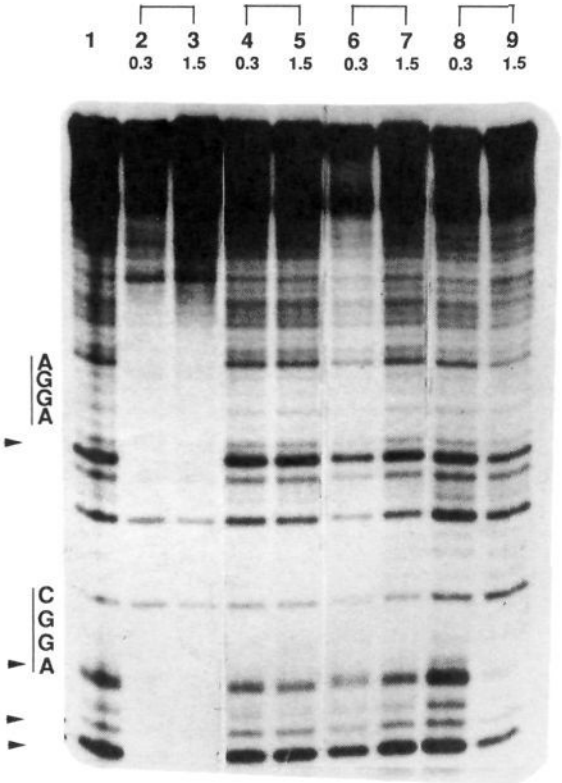

Figure 2. Inhibition of calicheamicin γ_1^I cleavage by carbohydrates: autoradiogram of 10% denaturing polyacrylamide gel showing the effect of preincubation with oligosaccharide on calicheamicin γ_1^I-mediated DNA cleavage. The DNA duplex ^{32}P-labeled at the 3′ end of the bottom (homopurine) strand was preincubated with 0.3 mM (lanes 2, 4, 6, 8) or 1.5 mM (lanes 3, 5, 7, 9) sugar for 15 min at 25 °C, followed by addition of calicheamicin γ_1^I (15 μM) for 15 min. Dithiothreitol (2 mM) was then added to initiate the drug cleavage reaction, and incubation was continued for 20 min. All reactions were carried out in 50 mM Tris·HCl (pH 8.0), 2% ethanol (v/v), 2% THF (v/v). DNA in each reaction was ethanol-precipitated, resolved by gel electrophoresis, and autoradiographed. The sugars used were as follows: lane 1, DNA + calicheamicin γ_1^I alone, no sugar; lanes 2 and 3, oligosaccharide **2**; lanes 4 and 5, sucrose; lanes 6 and 7, oligosaccharide **4**; lanes 8 and 9, oligosaccharide **3**. Calicheamicin γ_1^I binding sites and some prominent cleavage sites are indicated on the left.

pair selective, DNA minor groove binding drug, shows such a conformational perturbation at its binding site.[19] It is noteworthy that bases which are rendered accessible to cleavage by free aglycon due to oligosaccharide-induced local perturbation of DNA structure may not be cleaved by the tethered, conformationally restricted aglycon moiety of DNA-bound calicheamicin γ_1^I.

Thus, among the saccharides examined, it is shown that the aryl tetrasaccharide domain mimic (**2**) of calicheamicin γ_1^I possesses unique structural information which enables it to effectively compete against natural drug–DNA binding and cleavage (see Figure 2). When bound to duplex DNA, compound **2** may induce a local conformational perturbation, which leads to strong aglycon or Fe-MPE cleavage at positions which flank regions which are protected by the sugar (see Figure 1). The protected bases span natural drug-binding sites as well as neighboring positions, with relatively weak protection at the drug-binding sites.

It seems likely that precise positioning of the tethered aglycon in the drug is necessary to provide complementarity of fit between the oligosaccharide domain and the DNA minor groove at a specific sequence.[6] Absence of the tether apparently leads to modest displacements of the oligosaccharide to neighboring sites along the DNA duplex. It appears that the ability of the aglycon analog and Fe-MPE to access sites adjacent to bound oligo-

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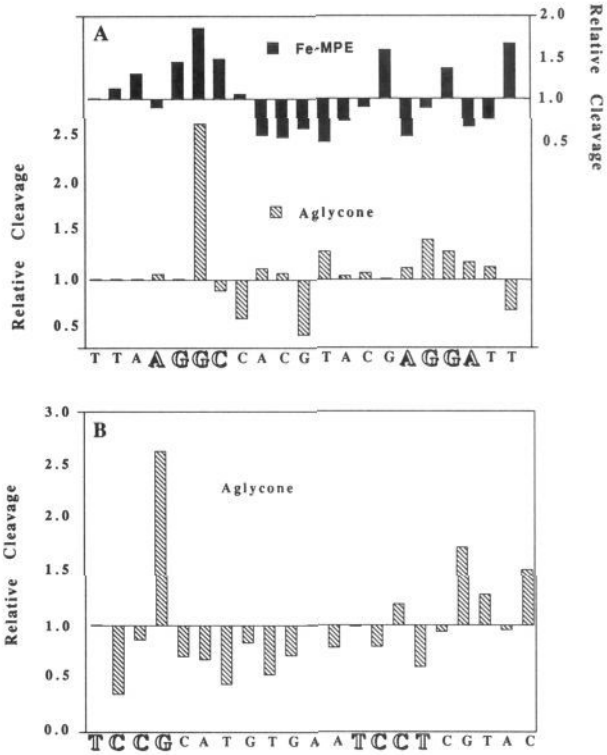

Figure 3. Densitometric analysis of oligosaccharide **2** induced differences in the susceptibility of DNA to cleavage by aglycon **5** and Fe-MPE reagents. (A) Cleavage of calicheamicin γ_1^I pentasaccharide-bound DNA (bottom strand): quantitation of the Fe-MPE and aglycon **5** bottom-strand-labeled footprinting experiments carried out in replicate. Oligosaccharide (0.2 mM) and DNA duplex were preincubated in 10 mM Tris·HCl (pH 8.0), 1 mM EDTA, 2% THF for 15 min at 25 °C. To one set of reactions was added Fe-MPE (10 μM), and incubation continued for 30 min. Addition of dithiothreitol (2 mM) initiated the cleavage reaction, which was continued for 40 min. To another set of reactions was added aglycon **5** (3.2 mM), and incubation continued for 2 h. DNA in each reaction was ethanol-precipitated, resolved by electrophoresis on a 10% denaturing polyacrylamide gel, and autoradiographed. All reactions were performed within the limit of one cut per DNA fragment (>85% uncut DNA). Autoradiograms were scanned using a Model 1650 scanning densitometer (Hoefer). Data sets corresponding to oligosaccharide-bound lanes were compared with data sets corresponding to DNA treated with cleaving reagent alone after normalization (multiplication by a factor of 0.8–1.2) to account for differences in intensity. The vertical scales on both sides represent reagent-mediated cleavage of oligosaccharide-bound DNA vs DNA alone. It is given by f_b/f_u, where f_b is the fractional cleavage at any base in the presence of 0.2 mM oligosaccharide, and f_u is the fractional cleavage by the reagent at the same base, but in the absence of bound sugar. Values >1 indicate enhancement, <1 indicate protected sites. Calicheamicin γ_1^I four-base recognition sites on the DNA are indicated in larger type than the rest of the sequence, shown at the bottom of the plot. (B) Cleavage of calicheamicin γ_1^I pentasaccharide-bound DNA (top strand): same as A, but quantitation of the aglycon **5** footprinting experiments with 0.2 mM oligosaccharide **2** on top-strand-labeled DNA duplex.

saccharide is limited, leading to broader footprints. Footprinting analyses are being pursued in greater detail to obtain more information on sequence selectivity and groove binding of the oligosaccharide.

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Supplementary Material Available: Available autoradiogram pertinent to Figure 3 with protocols (2 pages). Ordering information is given on any current masthead page.