DNA–Carbohydrate Interactions. Specific Binding of Head-to-Head and Head-to-Tail Dimers of the Calicheamicin Oligosaccharide to Duplex DNA

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · MARCH 1996

Impact Factor: 12.11 · DOI: 10.1021/ja9538142

6 AUTHORS, INCLUDING:

Keiichi Ajito
Meiji Holdings Co., Ltd.
56 PUBLICATIONS **699** CITATIONS

SEE PROFILE

DNA−Carbohydrate Interactions. Specific Binding of Head-to-Head and Head-to-Tail Dimers of the Calicheamicin Oligosaccharide to Duplex DNA

K. C. Nicolaou,* B. M. Smith, K. Ajito, H. Komatsu, L. Gomez-Paloma, and Y. Tor

Department of Chemistry, The Scripps Research Institute
10666 North Torrey Pines Road
La Jolla, California 92037
Department of Chemistry and Biochemistry
University of California, San Diego
9500 Gilman Drive, La Jolla, California 92093

Received November 13, 1995

As part of our program in the area of molecular recognition in biological systems,[1] we have recently designed and synthesized two dimeric forms of the calicheamicin γ_1^I oligosaccharide (see Figure 1, **1** and **2**), the head-to-head dimer **3** (hh dimer **3**),[2] and the head-to-tail dimer **4** (ht dimer **4**).[3] In this communication we wish to report the specific, high-affinity binding of the two dimeric oligosaccharides **3**[4] and **4** to certain duplex DNA sequences as determined by competitive binding experiments.

Molecules **3** and **4** were designed by computer modeling using information previously derived in these laboratories by ^{1}H NMR and competitive binding and footprinting experiments with calicheamicin γ_1^I oligosaccharide monomer **2** bound to 5′-TCCT·AGGA-3′ DNA binding sites.[5−7] These modeling studies revealed a good fit for compounds **3** and **4** into the minor groove of duplex DNA along the sequences 5′-AGGAXXTCCT·TCCTXXAGGA-3′ and 5′-TCCTTCCT·AGGAAGGA-3′, respectively (see computer-generated molecular models, Figure 2). These designed complexes were confirmed by using calicheamicin γ_1^I (**1**) as a cleaving agent in competitive binding experiments, which further demonstrated affinities of the dimers **3** and **4** to the targeted DNA sequences of more than 100-fold and 1000-fold higher, respectively, compared to the monomeric oligosaccharide **2**.

Synthetic 28-mers of duplex DNA containing the two binding sites 5′-AGGATGTCCT·AGGACATCCT-3′ and 5′-TCCTTCCT·AGGAAGGA-3′, 5′-^{32}P-labeled on one strand, were incubated with the oligosaccharides **2**, **3**, or **4** and calicheamicin γ_1^I (**1**) at 37 °C for 1 h. 2-Mercaptoethanol was then added to cause DNA cleavage, generating the cleavage patterns shown in Figures 3 and 4. As seen from the autoradiodiagram, calicheamicin γ_1^I cleaves DNA primarily at the expected binding sites (two 5′-TCCT·AGGA-3′ sites, see arrows, Figures 3 and 4)[8,9] but the cleavage is strongly inhibited by oligosaccharides **3** (Figure 4, lanes 5−12) and **4** (Figure 3, lanes 5−12). Comparisons with oligosaccharide monomer **2** revealed more than 100-fold higher inhibitory activity for the head-to-head dimer **3** and more than 1000-fold higher inhibitory activity for the head-to-tail dimer **4** as compared to the monomer **2**. Furthermore, cross-inhibition experiments (Figure 3, lanes 17−23, and Figure 4, lanes 17−22) demonstrated that each dimer prefers its own binding site by a factor of >100. These comparisons led to an estimated value of 10^8−10^9 for the binding constants of dimers **3** and **4** with their respective binding sites.[10]

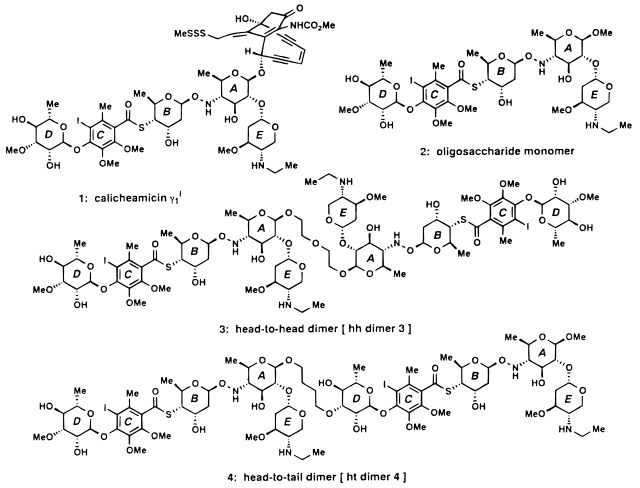

Figure 1. Structures of calicheamicin γ_1^I (**1**) and calicheamicin γ_1^I oligosaccharides **2**−**4**.

In addition to the strong and specific binding of the dimers to their targeted sequences, a number of interesting observations are worthy of comment. For example, while the inhibition of DNA cleavage by the ht dimer **4** is essentially the same for the two TCCT·AGGA binding sites (Figure 3, lanes 5−12), the effects of both the monomer **2** and the hh dimer **3** appear to be uneven for the two TCCT·AGGA sites (Figure 3, lanes 13−16 and 17−23). Moreover, the ratios of inhibition at the two contiguous sites are approximately reversed in the two cases (see arrows, Figure 3, lanes 5−12, 13−16, and 17−23). These phenomena may reflect the dependence of binding to each four-base sequence on flanking sequences.[11] It is noteworthy that such effects are not apparent in the case of the 5′-AGGATGTCCT-3′ containing 28-mer (see arrows, Figure 4, lanes 5−12, 13−16, and 17−22).

These results demonstrate striking site selectivities and affinities for oligosaccharide−DNA interactions and point the way for further experiments including (a) attachment of properly docked enediynes and other DNA-cleaving moieties to form specific DNA-cleaving agents; (b) design of higher homologs of these dimers to achieve even higher sequence specificity in DNA recognition; (c) generation of combinatorial libraries of oligosaccharides or DNA oligomers for screening against specific DNA sequences or oligosaccharides, respectively, in order to identify high-affinity matching pairs of molecules from these classes; and (d) studies in gene

(1) Nicolaou, K. C.; Theodorakis, E. A.; Claiborne, C. F. *Pure Appl. Chem.*, in press.
(2) Nicolaou, K. C.; Ajito, K.; Komatsu, H.; Smith, B. M.; Li, T.; Egan, M. G.; Gomez-Paloma, L. *Angew. Chem., Int. Ed. Engl*. **1995**, *34*, 576.
(3) Nicolaou, K. C.; Ajito, K.; Komatsu, H.; Smith, B. M.; Bertinato, P.; Gomez-Paloma, L. Submitted for publication in *Angew. Chem.*
(4) Previous binding studies of the head-to-head dimer **3** with duplex DNA containing 5′-TCCTATAGGA-3′ as a target sequence led to inconclusive data owing to the nonideal nature of this binding sequence as opposed to the one employed in the present study: see ref 2.
(5) Li, T. ; Zeng, Z.; Estevez, V. A.; Baldenius, K.-U.; Nicolaou, K. C.; Joyce, G. F. *J. Am. Chem. Soc.* **1994**, *116*, 3709.
(6) Gomez-Paloma, L.; Smith, J. A.; Chazin, W. J.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1994**, *116*, 3697.
(7) Nicolaou, K. C.; Tsay, S.-C.; Suzuki, T.; Joyce, G. F. *J. Am. Chem. Soc*. **1992**, *114*, 7555.
(8) Zein, N.; Sinha, A. M.; McGahren, W. J.; Ellestad, G. A. *Science* **1988**, *240*, 1198.
(9) While the restriction enzyme cuts shown in Figures 3 and 4 are sharp due to clean phosphate ester cleavages (free 3′-hydroxyl fragments), those caused by calicheamicin γ_1^I are often double, owing to the presence of 3′-phosphate and 3′-phosphoglycolate fragments, both of which migrate faster on the gel; see: Dedon, P. C.; Salzberg, A. A.; Xu, J. *Biochemistry* **1993**, *32*, 3617.
(10) The binding constants for calicheamicin γ_1^I and monomer **2** were previously determined to be 0.7×10^7 ($K_D = 0.135$ μM) and 2.4×10^5 ($K_D = 4.1$ μM), respectively; see ref 5.
(11) Zein, N.; Poncin, M.; Nilakantan, R.; Ellestad, G. A. *Science* **1989**, *244*, 697.

0002-7863/96/1518-2303$12.00/0

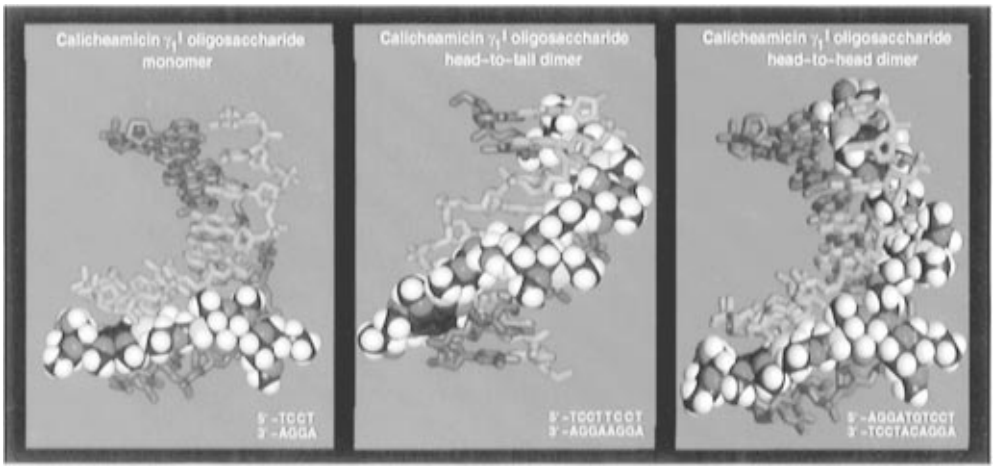

Figure 2. Computer-generated molecular models of oligosaccharide−DNA complexes. Left: monomer **2** bound to 5′-TCCT-AGGA-3′. Center: ht dimer **4** bound to 5′-TCCTTCCT·AGGAAGGA-3′. Right: hh dimer **3** bound to 5′-AGGATGTCCT·AGGACATCCT-3′. The DNA strands are displayed in green and red. The oligosaccharide atoms are colored according to the following code: carbon, red; hydrogen, white; oxygen, red; nitrogen, blue; sulfur, yellow; iodine, purple. Modeling studies and interactive docking were done on a SGI Indigo-2 workstation using Insight II (Biosym Technologies, Inc., San Diego, CA). Pictures were created using AVS (AVS Inc., Waltham, MA) and locally developed modules running on a DEC Alpha 3000/500 with a Kubota Pacific Denali graphics card.

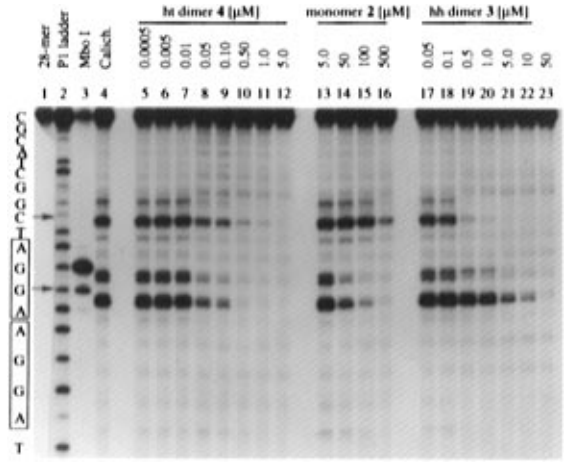

Figure 3. Autoradiodiagram of denaturing polyacrylamide gel showing products of calicheamicin-induced DNA cleavage of [5′-^{32}P]-labeled AGGAAGGA-containing DNA duplex in the presence of oligosaccharides **2** (monomer **2**), **3** (hh dimer **3**) or **4** (ht dimer **4**). Lane 1: 28-mer. Lane 2: 28-mer ladder resulting from partial digestion with nuclease P1; Lane 3: marker lane containing the products of DNA digestion with *Mbo*I restriction enzyme. Lanes 4−23: products of DNA cleavage with calicheamicin (2.5 μM) alone (lane 4) and in the presence of varying concentrations of head-to-tail dimer **4** (lanes 5−12), monomer **2** (lanes 13−16), and head-to-head dimer **3** (lanes 17−23). DNA cleavage by calicheamicin was performed as follows: the [5′-^{32}P]-labeled CGCATCGGCTAGGAAGGATCGGCTACGC DNA oligomer and its unlabeled complementary strand (10-fold excess) were hybridized in a buffered solution containing 100 mM NaCl and 1 mM Tris·HCl (pH 7.5) by incubation at 95 °C for 3 min and then slow cooling to 25 °C over 1 h. An aliquot of the hybridization mixture was added to a reaction mixture containing 20 mM NaCl, 30 mM Tris·HCl (pH 7.5), 10% DMSO (v/v), 2.5 μM calicheamicin, and varying concentrations of oligosaccharide **2**, **3**, or **4** in a final volume of 40 μL, and the mixture was preincubated at 37 °C for 1 h. Calicheamicin-dependent DNA cleavage was initiated by addition of 2 μL of 2-mercaptoethanol. Aliquots of 6 μL of reaction mixture were transferred to Eppendorf tubes. An equal volume of gel loading buffer containing 8 M urea and 0.05% (w/v) of both bromophenol blue and xylene cyanol was added to each tube, and the mixture was heated at 95 °C for 3 min (to effect denaturization) and then loaded on the gel. Tris = tris(hydroxymethyl)aminoethane. DMSO = dimethyl sulfoxide.

regulation and gene therapy via inhibition of transcription factor binding.[12,13]

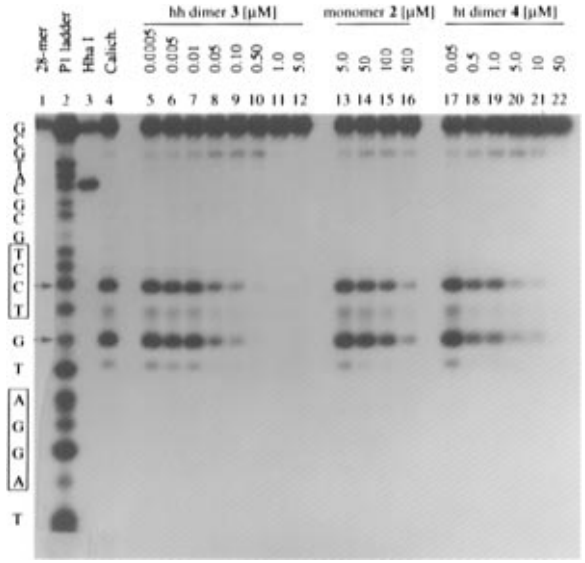

Figure 4. Autoradiodiagram of denaturing polyacrylamide gel showing products of calicheamicin-induced DNA cleavage of [5′-^{32}P]-labeled CGTAGCCGTAGGATG-TCCTGCGCATGCG DNA duplex in the presence of oligosaccharides **2** (monomer **2**), **3** (hh dimer **3**), and 4 (ht dimer **4**). Lane 1: 28-mer. Lane 2: 28-mer ladder resulting from partial digestion with nuclease P1. Lane 3: marker lane containing the products of DNA digestion with *Hha*I restriction enzyme. Lanes 4−22: products of DNA cleavage with calicheamicin (2.5 μM) alone (lane 4) and in the presence of varying concentrations of dimer **3** (lanes 5−12), monomer **2** (lanes 13−16), and dimer **4** (lanes 17−22). Methods were the same as in Figure 3.

Acknowledgment. We thank Dr. G. F. Joyce for helpful discussions and useful suggestions, Drs. Bill Marshal, Burt Goodman, and Ted Jones of Amgen (Boulder, CO) for generous samples of oligonucleotides, and Dr. George Ellestad of Lederle Laboratories for generous samples of calicheamicin γ_1^I. This work was financially supported by the National Institutes of Health, the ALSAM Foundation, Merck, Pfizer, Hoffmann La Roche, Schering-Plough, Amgen, and fellowships from Meiji Seika Kaisha, Ltd. (visiting scientist, K.A.), and Mitsui Toatsu Chemicals, Inc. (visiting scientist, H.K.).

JA9538142

(12) Liu, C.; Smith, B. M.; Ajito, K.; Komatsu, H.; Gomez-Paloma, L.; Li, T.; Theodorakis, E. A.; Nicolaou, K. C.; Vogt, P. K. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 940.

(13) Ho, S. N.; Boyer, S. H.; Schreiber, S. L.; Danishefsky, S. J.; Crabtree, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9203.