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NMR Characterization of Calicheamicin $\gamma_1{}^I$ Bound to DNA

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Abstract: Calicheamicin $\gamma_1{}^I$ is a diyne-ene antitumor antibiotic that binds preferentially to pyrimidine-rich sequences of DNA such as TCCT, ACCT, TCTC, and TTTT. We are using NMR spectroscopy to study the structure of calicheamicin bound to different DNA duplex octamers in order to shed light on the molecular basis for selective recognition. We have found that calicheamicin forms a unique and stable 1:1 complex with a duplex containing an ACCT recognition site. The tetrasaccharide-aryl tail is centered over the CpC step of the recognition sequence and the aglycone is positioned to abstract hydrogen atoms from the presumptive cleavage sites on this duplex. Binding induces significant conformational changes in the DNA but not, evidently, in the drug. The conformational changes appear to be larger in the pyrimidine strand than the purine strand. One possible explanation for this is that the bound drug, which does not lie symmetrically in the minor groove, exerts greater steric presssure on the pyrimidine strand than the purine strand. Calicheamicin also forms a unique 1:1 complex with a duplex containing a TTTT recognition site, and an analysis of the spectral data shows that it binds to the TTTT recognition site in the same orientation as it does to the ACCT recognition site. Moreover, it induces similar conformational changes in the pyrimidine strand. On the basis of the NMR results, we have proposed that the binding site selectivity of calicheamicin is due to the ability of pyrimidine/purine runs to adapt more readily than other sequences to the particular shape of the drug.

Introduction

Many antitumor agents act by binding in the minor groove of DNA and, in some cases, by damaging the DNA.[1] Small molecule-minor groove binding interactions are thus of great interest since a detailed understanding of them may ultimately lead to the ability to design new antitumor drugs.

The diyne-ene antibiotics are a new class of antitumor agents that rearrange to form diradicals that damage DNA.[2-7] A great deal of attention has been focused on understanding their cleavage chemistry and on designing synthetic diyne-enes with the ability to rearrange to form diradicals under specific conditions.[8] However, since the first step in DNA damage is DNA binding, equal attention should be paid to the binding components of the diyne-enes and how binding is related to activity. The diyne-enes are a structurally diverse family of compounds that utilize both intercalative and groove binding modes and display a wide range of affinities and selectivities. It is not yet understood how the DNA binding characterisitics of the various diyne-enes influence their antitumor activity. Nevertheless, the binding components of many of the diyne-ene antibiotics are interesting in their own right and a study of them may advance our understanding of small molecule-DNA recognition in general.

Calicheamicin $\gamma_1{}^I$ (Fig. 1) is a diyne-ene antitumor antibiotic that is also a site selective minor groove binder.[9,10] In this article we present results of NMR investigations of calicheamicin bound to different DNA sequences. We show that the pyrimidine recognition sites distort to provide a surface complementary to calicheamicin. Calicheamicin itself does not change conformation significantly upon binding. We propose that selective DNA recognition by calicheamicin is determined by a complex interplay of the shape of the drug and the ability of different DNA sites to adapt to that particular shape in a manner that provides the required level of stabilizing interactions.

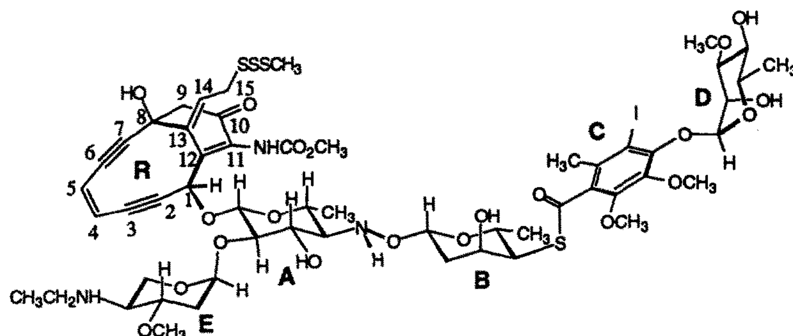
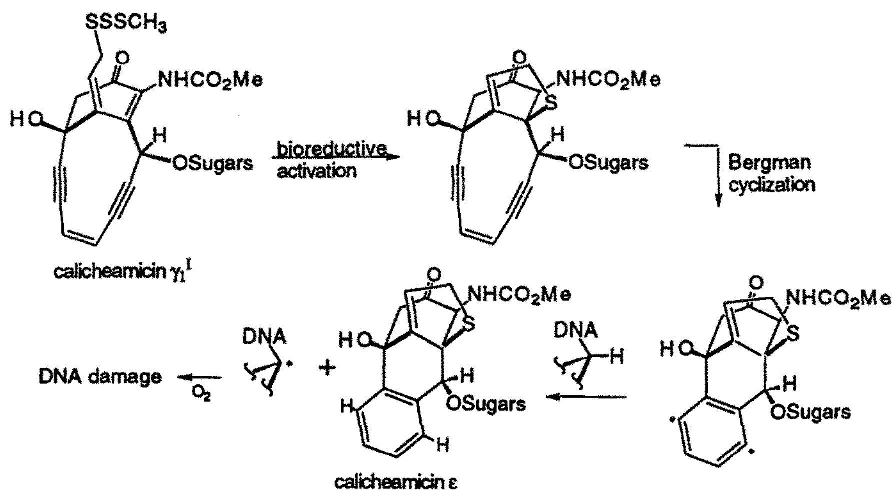


Fig. 1. Calicheamicin γ_1^I

Background

Like several other diyne-ene antibiotics, calicheamicin rearranges in the presence of thiols to produce a diradical that damages DNA (Scheme 1).[9,11] However, calicheamicin stands out among the diyne-ene antibiotics because it causes primarily double strand lesions and displays a higher degree of site selectivity.[9,12] The pattern of the lesions indicates that calicheamicin binds in the minor groove with the diradical positioned so that it is able to abstract a hydrogen atom from each DNA strand. Studies on derivatives of calicheamicin lacking various components have established that both the carbohydrate-aryl tail and the aglycone are critical for site selective minor groove binding.[13-15]



Scheme 1

Initial reports on the cleavage selectivity of calicheamicin identified TCCT, TCCC, TCTC, ACCT and a small number of other pyrimidine-rich GC-containing sequences as preferred binding sites. Various models were proposed to account for the sequence selectivity, with an emphasis placed on explaining the apparent requirement for GC base pairs in the recognition sequence.[16,17] In 1992, however, we reported DNA cleavage experiments showing that TTTT sequences are also good recognition sites for calicheamicin and in some cases

are cleaved in preference to GC-containing pyrimidine sequences such as TCCC.[14,10] Cleavage products suggested that calicheamicin binds to TTTT sites in a similar mode and with a similar orientation as it does to GC-containing pyrimidine tracts. This finding showed that there is no requirement for GC base pairs in the recognition sequence, and the focus changed from trying to understand why calicheamicin binds to pyrimidine runs containing GC base pairs to trying to understand why calicheamicin binds to pyrimidine-rich sequences in general. Since TTTT and GC-containing pyrimidine sequences are unlikely to have similar inherent conformations (because the presence of the exocyclic amino groups in the minor groove alters both the width of the groove and the immediate steric environment), we suggested that calicheamicin induces a conformational change in the DNA upon binding.[14] To get more insight into the calicheamicin-DNA interaction, we have undertaken NMR studies on calicheamicin bound to small DNA duplexes containing different sequences.[18]

Results

There were two separate issues we were concerned about at the onset of our NMR investigations. The first concern was whether we would be able to obtain interpretable data. For this we needed calicheamicin to be chemically stable for prolonged periods in the presence of DNA and to bind to DNA with sufficient affinity and selectivity to form a unique 1:1 complex. No other NMR studies of any diyne-ene-DNA complexes have been reported, perhaps because most of the known diyne-ene antibiotics do not have the requisite characteristics. Our second concern was whether NMR studies on a calicheamicin-DNA complex would be relevant to understanding the molecular basis for the cleavage selectivity since the compound that effects DNA cleavage is not calicheamicin itself, but an intermediate diradical (see Scheme 1) that might bind differently than the parent compound.

To determine the feasibility of NMR studies on calicheamicin bound to DNA, we titrated a small DNA duplex containing a recognition site with increasing amounts of calicheamicin. The duplex, d[$G_1T_2G_3A_4C_5C_6T_7G_8$]-d[$C_9A_{10}G_{11}G_{12}T_{13}C_{14}A_{15}C_{16}$], was designed to have an ACCT recognition site, two base pairs at the 5' side of the recognition sequence to accommodate the aglycone, and a GC base pair at each end for stability. The presumptive cleavage sites are C5 H5' in the recognition sequence and A15 H4' in the flanking sequence on the opposite strand.[9,19,20]

One-dimensional 500 MHz ^{1}H NMR spectra of the DNA duplex alone and in the presence of 0.5 and 1.0 equivalents of calicheamicin are shown in Figure 2. Upon adding 0.5 equivalents of calicheamicin,

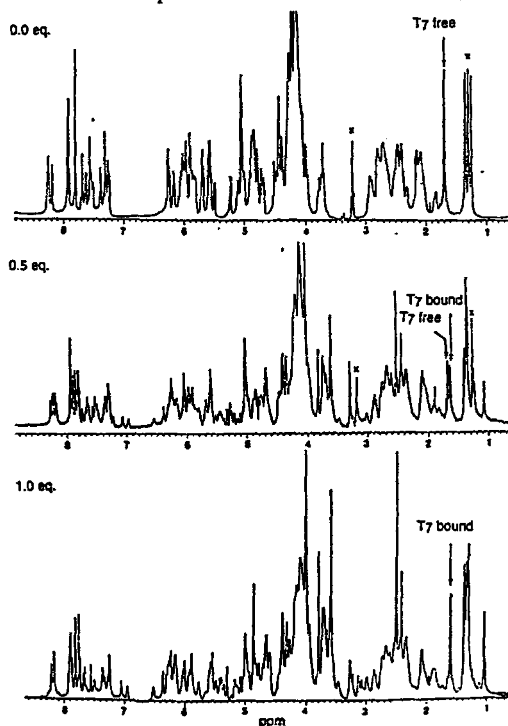


Figure 2. Titration of DNA with calicheamicin.

many of the DNA resonances double. This doubling, seen most clearly with the resolved thymine methyl resonance near 1.7 ppm (T7, Figure 2), shows that there are now two discrete sets of DNA resonances, corresponding to free and bound DNA octamers. The drug is thus in slow exchange between DNA octamers on the NMR chemical shift time scale. The free DNA resonances disappear completely upon the addition of 1 equivalent of calicheamicin. Moreover, the 0.5:1 and 1:1 complexes proved to be stable for an indefinite period because calicheamicin, unlike many other diyne-ene antibiotics (*e.g.*, neocarzinostatin), does not rearrange at a detectable rate in the absence of exogenous thiol. Thus, the titration experiments indicated that full assignments of a 1:1 calicheamicin-DNA complex would be possible. It remained to be established whether the structure of the complex formed between the parent diyne-ene and a short DNA duplex in solution would be relevant to understanding the site selective cleavage behavior of the activated intermediate in the presence of much longer pieces of DNA.

The non-exchangeable resonances of the free duplex and the 1:1 complex were assigned from NOESY, DQF-COSY, and TOCSY data and many intermolecular NOEs were identified (Tables 1, 2, and 3).[18] The NOEs show that calicheamicin binds in the minor groove with the tetrasaccharide-aryl tail centered over the CpC step of the ACCT recognition sequence (Figure 3). Although resonance overlap prevents unambiguous identification of intermolecular NOEs to the aglycone, the position of the aglycone can be inferred from the placement of the oligosaccharide tail and the orientation of the aglycone with respect to the oligosaccharide tail (Tables 3 and 4). When various NOE and conformational constraints are satisfied, the aglycone is positioned to abstract hydrogen atoms from the C5 and the A15 ribose sugars, the presumptive cleavage sites for this duplex. The orientation of the parent diyne-ene in the groove is thus similar to the orientation of the activated intermediate as determined from atom transfer experiments.[19,20,21] This finding is important because it implies that NMR studies on complexes formed between the parent diyne-ene and DNA are relevant to understanding the molecular basis for site selective cleavage by the activated intermediate.

Figure 3. Representation of calicheamicin bound to the DNA octamer $d[G_1T_2G_3A_4C_5C_6T_7G_8]$-$d[C_9A_{10}G_{11}G_{12}T_{13}C_{14}A_{15}C_{16}]$. The intermolecular NOEs are indicated by bold lines. The E ring ethylamino sugar, which protrudes from the groove and can form a salt bridge to the C5 phosphate, is not shown.

The bound conformation of calicheamicin was evaluated from DQF-COSY and NOESY spectra and compared to its solution conformation. COSY crosspeak intensities were analyzed for information on sugar ring

TABLE 1. ^1H Chemical Shifts (ppm) of the Non-Exchangeable DNA Resonances in the Calicheamicin-d[$G_1T_2G_3A_4C_5C_6T_7G_8$]-d[$C_9A_{10}G_{11}G_{12}T_{13}C_{14}A_{15}C_{16}$] Complex

	H6/H8	H2	H5	CH_3	H1'	H2'	H2"	H3'	H4'
G1*	7.92 (-0.02)				5.94 (0.00)	2.56 (+0.03)	2.75 (+0.01)	4.80 (+0.01)	4.21 (+0.05)
T2	7.27 (+0.02)			1.32 (+0.01)	5.79 (+0.01)	2.06 (+0.03)	2.42 (+0.03)	4.87 (+0.02)	4.18 (+0.03)
G3	7.89 (+0.01)				**5.37 (+0.19)**	2.67 (+0.02)	2.69 (+0.08)	4.99 (+0.03)	4.30 (+0.06)
A4	8.19 (-0.01)	7.85 (-0.06)			**6.54 (-0.31)**	2.69 (-0.02)	**3.12 (-0.21)**	5.05 (-0.03)	**4.34 (+0.16)**
C5	**6.98 (+0.26)**		5.10 (+0.10)		**5.89 (-0.11)**	**1.75 (+0.30)**	**2.87 (-0.45)**	4.84 (-0.08)	4.14 (+0.06)
C6	**7.35 (+0.14)**		5.41 (+0.05)		**5.61 (+0.27)**	**1.91 (+0.15)**	**2.63 (-0.20)**	**4.61 (+0.16)**	**4.61 (-0.46)**
T7	7.39 (-0.03)			1.62 (+0.05)	**6.05 (-0.21)**	**1.87 (+0.13)**	2.44 (-0.07)	**4.63 (+0.23)**	4.15 (+0.10)
G8	7.93 (-0.01)				6.17 (-0.02)	2.61 (+0.03)	2.32 (+0.05)	4.70 (0.00)	4.19 (+0.01)
C9*	7.69 (-0.07)		5.91 (-0.04)		5.56 (-0.03)	1.92 (-0.10)	2.35 (-0.06)	4.67 (+0.01)	4.03 (0.00)
A10	8.19 (+0.04)	7.78 (+0.01)			**6.29 (-0.32)**	2.70 (+0.06)	**3.08 (-0.18)**	5.02 (0.00)	4.42 (-0.04)
G11	7.58 (+0.09)				**5.50 (+0.17)**	**2.49 (+0.12)**	**2.52 (+0.15)**	4.96 (+0.02)	4.42 (0.00)
G12	**7.76 (-0.22)**				**6.15 (-0.21)**	2.47 (+0.01)	2.75 (-0.01)	**5.02 (-0.20)**	4.43 (-0.01)
T13*	**7.07 (+0.21)**			**1.05 (+0.18)**	**6.20 (-0.17)**	2.01 (+0.10)	2.52 (-0.01)	**4.73 (+0.12)**	**3.15 (+1.00)**
C14	7.52 (+0.01)		5.59 (+0.07)		5.56 (-0.01)	2.11 (-0.05)	2.36 (+0.02)	4.85 (-0.01)	4.04 (+0.09)
A15	8.23 (+0.02)	7.84 (-0.06)			6.26 (-0.03)	2.68 (0.00)	2.89 (-0.02)	5.01 (0.00)	4.41 (+0.02)
C16	7.26 (+0.03)		5.18 (-0.09)		6.02 (-0.02)	2.08 (+0.04)	2.09 (-0.03)	4.46 (-0.01)	3.97 (+0.01)

*H5' resonances for these nucleotides were also assigned: G1H5's = 3.71, 3.73 ppm; C9H5's = 3.71, 3.70 ppm; T13H5's = 3.26, 3.58 ppm. Shift changes upon complexation are shown in parentheses: difference = d(free duplex) - d(complex). Absolute chemical shift changes >0.10 ppm are listed in bold type.

TABLE 2. ^1H Chemical Shifts (ppm) of Calicheamicin in the Complex

	H1	H2(a)*	H2(e)*	H3	H4	H5(a)*	H5(e)*	CH_3	NCH_2-	-CH_2CH_3	OCH_3(m)*	OCH_3(o)*
A	4.64	3.49		4.35	2.35	4.03		1.33				
B	5.16	1.89	2.36	4.34	3.72	4.12		1.38				
C								2.43			4.03	3.82
D	5.33		4.67	4.04	3.63	4.25		1.39			3.61	
E	5.45	1.64	2.76	4.06	3.30	4.06	4.13		3.01	1.36	3.61	
	H1	H4†	H5†	H9a	H9b	H14	H15a	H15b	SCH_3			
R	6.40	6.26	6.19	3.26	2.86	6.30	3.95	3.65	2.52			

*The parentheses specify the resonance in question when there is more than one possibility: a=axial, e=equatorial, referring to protons on a single carbon; o=ortho, m = meta, referring to the relationship of the methoxy group to the D ring glycosidic linkage. †Tentative. See Figure 1 for numbering of the aglycone.

TABLE 3. Intermolecular NOEs in the Calicheamicin-DNA Complex

calicheamicin residue*		$d[G_1T_2G_3A_4C_5C_6T_7G_8]$-$d[C_9A_{10}G_{11}G_{12}T_{13}C_{14}A_{15}C_{16}]$ DNA residue	H1'	H4'	H5',H5"
A sugar[a]	A1	C5		w	
	A3	C5	w		
	A6	A15		s	
B sugar[b]	B2a	C5	w		
	B2e	C5	w		
	B3	C6	w		
	B4	C6	w		
	B4	C6		m	
	B6	C6		m	
C ring[c]	CH3	C6	s		
	OCH3(meta)†	T13		m	
D sugar[d]	D1	T13		w	m
	D2	G12		w	
	OCH3	G12		m	

(s, strong; m, medium; w, weak). †Meta to the D ring glycosidic linkage. *Intermolecular NOEs from the aglycone R1, R4 and R5 cannot be unambiguously assigned because of resonance overlap; (a) the A sugar of calicheamicin spans the minor groove, contacting the A15 and C5 backbone sugars on opposite strands of the DNA; (b) the b sugar contacts the C5 and C6 sugars on the pyrimidine strand; (c) the C ring contacts the C6 and T13 sugars on opposite strands of the DNA; (d) the D sugar contacts the G12 and T13 sugars on the purine strand.

conformation while NOESY crosspeak intensities were analyzed for information on the orientation of the residues with respect to each other and to the floor of the minor groove. The spectral data indicate that the bound conformation of calicheamicin is very similar to the average solution conformation. For example, large (~9 Hz) vicinal coupling constants and correspondingly large COSY crosspeaks show that the A ring of calicheamicin is in the $^4C_1$ (chair) conformation in solution. When the drug is bound to DNA, the relative intensities of the COSY crosspeaks for the A ring are similar to what they are in solution, indicating that the conformation does not change significantly. The relative COSY crosspeak intensities for the other sugars are also similar in solution and bound to DNA. Thus, each sugar ring maintains its preferred conformation upon binding. Moreover, the same interresidue NOEs are observed in the complex (Table 4) and in the unbound drug. Based on NMR data for calicheamicin in solution, we have previously argued that calicheamicin is substantially preorganized for binding because it consists of rigid subunits (the aglycone, the hexose sugars and the aromatic ring) connected by bonds which mostly have well defined conformational preferences.[22] We proposed that the unusual hydroxylamine glycosidic linkage is a critical feature in determining the orientation of the two halves of the oligosaccharide-aryl tail and therefore the overall shape of the molecule.[23] In the calicheamicin-DNA complex there is a strong NOE between the B1 proton and the A6 methyl which, in conjunction with intermolecular NOES from the A and B rings to the DNA, shows that the bound conformation around the hydroxylamine glycosidic linkage is similar to the low energy solution conformation (identified using solution NMR data in conjunction with molecular modeling, and observed in a crystal structure of a fragment of calicheamicin).[23,24] We think that the relative rigidity and shape of calicheamicin, determined by such features

as the hydroxylamine glycosidic linkage, is intimately related to the proposed mechanism for site selective recognition (*vide infra*).

TABLE 4. Interresidue NOEs in Bound Calicheamicin.

residue 1	proton	residue 2	proton	size
aglycone	R1	A sugar	A1	m
	R4 (A15H1')[a]		A6	w
	R5		A6	w
	R1	E sugar	E5a (E3)[b]	m
	R1		E5e (C5H4')[c]	m
A sugar	A5	B sugar	B1	w
	A2	E sugar	E1	s
	A6		B1	m
B sugar	B3	C ring	CH3	w
C ring	OCH3(ortho)†	D sugar	D1	w
	OCH3(ortho)†		D6	m

(s, strong; m, medium; w, weak). † Ortho to the D ring glycosidic linkage. A few NOEs from the drug cannot be unambiguously assigned because of resonance overlap: (a) the downfield vinyl resonance overlaps with A15H1'; (b) the E5a and E3 resonances overlap in the complex; NOEs from R1 to both resonances are observed in the free drug; (c) the E5e resonance overlaps with C5H4'.

The NMR spectra of the complex also provide information on the conformation of the DNA. Upon binding calicheamicin the DNA remains in a right-handed conformation with many features characteristic of B form DNA. However, both NOESY and COSY spectra provide evidence for a notable conformational change in the CpC step of the recognition sequence. For example, in a typical B DNA duplex in solution, the COSY crosspeaks between the H1' and H2' ribose sugar protons are larger than those between H1' and H2", reflecting the relative sizes of the three bond couplings (*i.e.*, $^3J_{H1'-H2'} > {}^3J_{H1'-H2''}$ for the C2'-endo sugar conformation typically observed in B DNA).[25] All the ribose sugars in the free DNA duplex fit the pattern expected for B form DNA. In the bound duplex, however, the COSY crosspeak between C6 H1' and C6 H2" is present while that between C6 H1' and C6 H2' is not.[18] Moreover, another deviation from the standard pattern *both* 2 deoxy protons have a COSY crosspeak to the H3' proton. The C6 ribose sugar has thus undergone a change to a conformation with a pseudorotation angle less than 90°, which is closer to the type of sugar pucker found in A form DNA than B form DNA. Unusual NOE intensities between C6H6 and both the C6 and C5 2-deoxy protons provide support for a conformational change in the CpC step. The conformational changes indicate that the groove has to open up to accommodate the drug. We were interested to find that both the NOEs and sugar coupling constants for the DNA suggest that the pyrimidine strand undergoes more extensive changes upon binding calicheamicin than the complementary purine strand. One possible explanation for this is that the bound drug exerts greater steric pressure on the pyridmidine strand than the purine strand. The NMR data show that the conformation of calicheamicin is such that the oligosaccharide-aryl tail does not lie symmetrically between the walls of the groove along its length, but curves down from the aglycone towards the pyrimidine strand and then back up towards the purine strand. Several intermolecular NOEs show that the B sugar and the C ring methyl of calicheamicin lie very close to the C6 ribose sugar of the DNA while the D sugar is very close to G12 and T13 on the purine strand (see Fig. 3).

Since calicheamicin cleaves both GC containing pyrimidine tracts and TTTT tracts, we were interested to see whether it would bind in the same orientation to both types of sequences. We have recently assigned the resonances and analyzed the structure of the complex formed between calicheamicin and an octamer with the sequence d[GCGTTTTG]-d[CAAAACGC], where TTTT is the intended recognition sequence.[26] The results show that calicheamicin binds to this sequence in exactly the same orientation as it does to the sequence containing the ACCT recognition site. Moreover, COSY spectra of the complex indicate a significant conformational change in the T6 ribose sugar of the pyrimidine strand. Intermolecular NOEs indicate that the B sugar and part of the C ring of calicheamicin lie very close to the T6 ribose sugar, perhaps exerting steric pressure on the pyrimidine strand. Thus, although the ACCT and TTTT recognition sites differ significantly in inherent shape (because one contains bulky amino groups in the groove), calicheamicin binds in a very similar fashion and appears to induce similar conformational changes in the pyrimidine strands of both sequences.

We have also looked at calicheamicin bound to the octamer d[CAGTCACG]-d[CGTGACTG], which was identified from a pUC19 NdeI-AccI restriction fragment as a sequence that is not cleaved by calicheamicin.[14] When one equivalent of calicheamicin was added to this DNA octamer, we observed at least three different sets of drug peaks in slow exchange on the NMR time scale. This means that there are at least three different binding modes for calicheamicin on this short DNA duplex. The fact that calicheamicin is in slow exchange between binding sites implies a relatively high affinity even for non-recognition sites. Calicheamicin is thus a good general DNA groove binder, a fact that is not surprising since it is a relatively rigid hydrophobic molecule with an extended conformation roughly complementary to the shape of the groove. The preference for pyrimidine-rich regions of DNA probably amounts to only a very few kilocalories.

Discussion

Extensive studies on different classes of natural products are beginning to shed light on minor groove recognition and the factors that determine selectivity.[27] The picture that is emerging from these studies is that shape selection is the principal means by which minor groove binders recognize particular sites. Most minor groove binders are relatively rigid molecules that bind to DNA sites that provide a complementary fit. *The complementary fit may or may not involve a significant conformational reorganization on the part of the DNA.* Thus, sequence dependent DNA flexibility - i.e. the ability of certain DNA sequences to adapt to the shape of a particular ligand at a relatively low energy cost- may play a large role in the binding selectivity of some minor groove binders.

Until recently, there has been a tendency to categorize minor groove binders as either AT-selective binders or GC-selective binders, in part because several examples of both types of ligands are known and in part because AT-rich and GC-rich sequences are viewed as conformational extremes.[1,28] The presence or absence of an exocyclic guanine amino group in the minor groove influences both the immediate steric environment at the floor of the groove and the local groove width. Netropsin and other planar aromatic ligands bind preferentially to AT-rich regions of DNA where they can penetrate deeply into the minor groove and gain stabilization through close association with the walls of the narrow groove without requiring a large conformational reorganization of the DNA.[29] Such a ready fit is not possible at GC containing sites which have both a wider groove and a bulky amino group which prevents deep penetration. In contrast, the bulky chromomycin dimer binds to GC-rich regions of DNA (e.g., GpG) and induces a dramatic conformational change from B form to A form DNA.[30]

The GC selectivity appears to be due in part to the fact that some steps (*i.e.*, GpG) undergo a B to A transition at lower cost than other steps,[31] and in part to the fact that guanines have amino groups that can replace solvent hydrogen bonds to the phenolic hydroxyls of the chromomycin dimer upon binding.

The cleavage data on calicheamicin showed that it is an unusual minor groove binder because it defies the usual AT-selective vs. GC-selective categorization. Instead, it is selective for pyrimidine-rich sequences almost regardless of base composition. Our NMR results show that calicheamicin has a relatively high affinity for duplex DNA in general, but forms specific complexes with DNA sequences containing pyrimidine recognition sites. We have shown that calicheamicin binds to two very different pyrimidine-rich recognition sites (ACCT and TTTT) in exactly the same orientation, inducing similar conformational changes in the pyrimidine strands of both sequences. The conformation of calicheamicin itself does not appear to change significantly upon binding to either sequence. We therefore suggest that the binding site selectivity of calicheamicin is due to the ability of pyrimidine/purine runs to adapt with a lower energy cost than other sequences to the particular shape of the drug. There is evidence that pyrimidine/purine runs have some conformational peculiarities that may relate to the differential energetics of stacking of the bases in the two strands.[32] Since the calicheamicin oligosaccharide curves down from the aglycone to exert greater steric pressure on the pyrimidine strand than the purine strand, we wonder whether pyrimidine/purine tracts are somehow able to adjust more readily to differential steric pressures than mixed sequences of DNA. Perhaps the pyrimidine strand can come partially unstacked to accommodate calicheamicin binding and/or increased stacking along the purine strand can help pay the cost of opening up the groove. If the binding site selectivity reflects the ability of certain sequences to adapt more readily than others to the steric demands of the drug, one might expect binding to be influenced by flanking sequences. Indeed, it has been reported that flanking sequences have a significant effect on the probability of cleavage at a particular recognition site.[17] With the aid of molecular modeling, we are currently refining the structures of the specific calicheamicin-DNA complexes reported herein. The refined structures should provide additional insight into the molecular basis for binding selectivity.

Experimental

Purified calicheamicin was a gift from Lederle Laboratories. The octanucleotides were synthesized on a 10 mmol scale at the Princeton Synthesis Facility and dialyzed to remove impurities. After lyophiliation, each strand was dissolved in 0.22 mL NMR buffer (10 mM sodium phosphate, ph 7.0/70 mM NaCl/0.05 mM EDTA), and the concentrations were determined from the measured absorbances and calculated extinction coefficients. Equimolar amounts of the complementary strands were mixed and the volume of the sample was brought to 0.5 mL with NMR buffer containing sodium 3-(trimethylsilyl)-1-propane-sulfonate as an internal reference. After annealing and repeated lyophilization from D_2O, the samples were dissolved in 1.0 mL D_2O and calicheamicin was added in ~0.5 mL CD_3OD. The samples were transferred to amber NMR tubes where the volume was reduced to 0.5 mL by evaporation under argon. The concentration of the DNA was 3 mM.

^{1}H NMR experiments were recorded on a JEOL GSX/GX 500 MHz spectrometer. NOESY experiments were acquired at 15 °C, with 2048 complex data points in the t2 dimension and 300-500 data points in the t1 dimension. Mixing times ranged from 50 to 200 ms. DQF-COSY experiments were recorded at both 15 °C and 21 °C, with 2048 complex data points and 370 t1 increments. Phase sensitive TOCSY spectra were recorded

using the MLEV-17 spin-lock pulse with mixing times of 50 and 65 ms. The data were processed using the FELIX program (Hare Research, Inc.).

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