Methyl (1α,3aβ,7aα)-2-(Bromomethyl)-1-ethyloctahydro-4-oxo-3a*H*-indene-3a-carboxylate (49a) and Methyl (1β,3aβ,7aβ)-2-(Bromomethyl)-1-ethyloctahydro-4-oxo-3a*H*-indene-3a-carboxylate (50a). A solution of methyl 2-bromo-2-oxo-2-(3-propenyl)dec-7(*Z*)-enoate (**48a**, 55.6 mg, 0.175 mmol) and hexamethylditin (9 μL, 0.027 mmol) in benzene-d_6 (0.6 mL) was irradiated for 1 h. The solvent was evaporated and the residue was purified by flash chromatography (10% EtOAc/hexane) to afford **49a/50a** (37.6 mg, 68%, **49a/50a** = 8.5/1 as determined by GC analysis): ^{1}H NMR (300 MHz, $CDCl_3$, **49a**) δ 3.68 (s, 3 H), 3.61 (dd, *J* = 9.7, 4.0 Hz, 1 H), 3.16 (dd, *J* = 9.7, 9.7 Hz, 1 H), 2.75-2.58 (m, 1 H), 2.55-2.35 (m, 3 H), 2.23 (dd, *J* = 14.4, 8.0 Hz, 1 H), 2.04 (dd, *J* = 14.4, 7.7 Hz, 1 H), 2.10-2.00 (m, 1 H), 1.85-1.75 (m, 2 H), 1.66-1.23 (m, 4 H), 0.98 (t, *J* = 7.4 Hz, 3 H); IR (thin film) 2951, 2866, 1712, 1452, 1429, 1248, 1172, 1105, 1030 cm^{-1}; HRMS calcd for $C_{14}H_{21}BrO_3$ (M^+) *m/e* 316.0674, obsd *m/e* 316.0674; LRMS *m/e* 316 (M^+), 286, 284, 259, 257, 237, 208, 195, 177. The structure of the minor isomer **50a** was assigned by its conversion to the known olefin **47**.

Methyl (1α,3aβ,7aα)-2-(Iodomethyl)-1-ethyloctahydro-4-oxo-3a*H*-indene-3a-carboxylate (49b) and Methyl (1β,3aβ,7aβ)-2-(Iodomethyl)-1-ethyloctahydro-4-oxo-3a*H*-indene-3a-carboxylate (50b). A solution of methyl 2-iodo-3-oxo-2-(3-propenyl)dec-7(*Z*)-enoate (**48b**, 130.0 mg, 0.357 mmol) and hexamethylditin (18 μL, 0.055 mmol) in benzene-d_6 (1.2 mL) was irradiated for 24 min. The solvent was evaporated and the residue was purified by flash chromatography (10% EtOAc/hexane) to afford **49b/50b** (91.2 mg, 70%, **49b/50b** = 10 as determined by GC analysis): ^{1}H NMR (300 MHz, $CDCl_3$, **49b**) δ 3.67 (s, 3 H), 3.44 (dd, *J* = 9.2, 3.7 Hz, 1 H), 2.87 (dd, *J* = 11.7, 9.3 Hz, 1 H), 2.71-2.57 (m, 1 H), 2.53-2.31 (m, 3 H), 2.29 (dd, *J* = 14.7, 8.2 Hz, 1 H), 2.08-2.02 (m, 1 H), 1.89 (dd, *J* = 14.7, 8 Hz, 1 H), 1.82-1.70 (m, 2 H), 1.68-1.54 (m, 1 H), 1.48-1.23 (m, 3 H), 0.97 (t, *J* = 7.6 Hz, 3 H); IR (thin film) 2955, 2870, 1716, 1456, 1252, 1192 cm^{-1}; HRMS calcd for $C_{14}H_{21}IO_3$ (M^+) *m/e* 364.0535, obsd *m/e* 364.0535; LRMS *m/e* 364 (M^+), 333, 332, 305, 263, 237, 205, 177, 159. The structure of the minor isomer **50b** was assigned by its conversion to the known olefin **47**.

Methyl (1α,3aβ,7aα)-1-Ethyl-2-methyleneoctahydro-4-oxo-3a*H*-indene-3a-carboxylate (46) and Methyl (1β,3aβ,7aβ)-1-Ethyl-2-methyleneoctahydro-4-oxo-3a*H*-Indene-3a-carboxylate (47). The bromides **49a/50a** (35.0 mg, 0.110 mmol) were dissolved in benzene-d_6 (0.7 mL), and DBU (46 μL, 0.307 mmol) was added. The tube was sealed, and the solution was heated at 120 °C for 4 h. After cooling, the tube was opened and the reaction mixture was applied to a silica gel plug and eluted with 30% EtOAc/hexane. Solvent removal afforded **46/47** (22.4 mg, 86%, **46/47** = 10), identical with an authentic sample prepared by Mn(III)-mediated cyclization of **45** by TLC, GC, and ^{1}H NMR comparison.

Methylenetetrahydrofurans 51a and 51b. When a sample of impure iodide **18** (containing about 15% unreacted **15**) was allowed to stand overnight in the dark at 25 °C and then purified by flash chromatography (20% EtOAc, hexane), methylenetetrahydrofuran **51a** was isolated in 40% yield as a yellow oil: ^{1}H NMR (C_6D_6, 300 MHz) δ 6.18 (m, 1 H), 5.33 (d, *J* = 17.1 Hz, 1 H), 5.13 (d, *J* = 9.8 Hz, 1 H), 3.54 (s, 3 H), 3.41 (d, *J* = 6.2 Hz, 2 H), 3.16-2.97 (m, 2 H), 2.69 (s, 2 H), 1.57 (ddd, *J* = 12.7, 9.3, 7.1 Hz, 1 H), 1.27 (ddd, *J* = 12.7, 8.2, 6.2 Hz, 1 H), 1.04 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.97, 169.12, 136.77, 114.15, 101.08, 86.50, 51.03, 34.57, 31.55, 30.29, 25.30, 14.16; IR (neat) 2990, 1699, 1635, 1313, 1182, 1115, 960 cm^{-1}, HRMS calcd for $C_{12}H_{17}IO_3$ *m/e* 336.0200, obsd *m/e* 336.0222; LRMS *m/e* 305, 227, 209, 177, 141. Standard tin hydride reduction of **51a** produced **51b**: ^{1}HMR (300 MHz, $CDCl_3$) δ 5.80 (m, 1 H), 4.97 (br d, *J* = 17.2 Hz, 1 H), 4.87 (br d, *J* = 9.8 Hz, 1 H), 3.66 (s, 3 H), 3.15 (d, *J* = 7.8 Hz, 2 H), 3.00 (d, *J* = 6.2 Hz, 2 H), 1.86 (t, *J* = 7.8 Hz, 2 H), 1.33 (s, 6 H); IR (neat) 2976, 1701, 1633, 1313, 1219, 1115 cm^{-1}; HRMS calcd for $C_{12}H_{18}O_3$ *m/e* 210.1256, obsd *m/e* 210.1256; LRMS *m/e* 179, 163, 154, 141, 122, 109.

Lactone Dihydrofuran 52. A solution of acetoacetate **15** (19.2 mg, 0.90 mmol) and molecular iodine (24.2 mg, 0.09 mmol) in benzene-d_6 was allowed to stand in the dark for 30 h. The mixture was diluted with Et_2O and then washed with aqueous $Na_2S_2O_3$, water, and brine. Drying and concentration provided 25.0 mg (85%) of a crude yellow oil identified as **52**. This crude product was rather pure (single peak on GC), but decomposed over several days at room temperature. Further purification was not attempted: ^{1}H NMR (300 MHz, C_6D_6) δ 4.04 (m, 1 H), 3.04 (br t, 2 H), 2.76 (m, 3 H), 2.41 (m, 1 H), 1.48 (s, 2 H), 1.34 (t, *J* = 7.2 Hz, 2 H), 1.02 (s, 3 H), 1.00 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) 172.00, 169.91, 92.50, 89.01, 75.24, 35.97, 31.85, 29.94, 27.23, 9.14; IR (neat) 2972, 1741, 1670, 1300, 1182, 987 cm^{-1}; LRMS *m/e* 322 (M^+) 279, 253, 195, 177, 145, 125.

Evidence for Hydrophobic Interaction between Calicheamicin and DNA

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Abstract: A hydrophobic interaction has been shown to be an important factor in the calicheamicin/DNA association. This is based on the effect of various inorganic salts on the rate of cleavage of covalently closed-circular form I DNA and the temperature dependence of the calicheamicin/DNA interaction. The strongly hydrated Na_2SO_4 increased the rate of cleavage by decreasing the solubility of the very lipophilic calicheamicin, thus strengthening the hydrophobic association with the DNA. The weakly hydrated $NaClO_4$ had the opposite effect since it increased the solubility of calicheamicin in the aqueous solution. The enthalpy change ($\Delta H°_{obs}$) for calicheamicin/DNA binding is temperature-dependent, and the negative heat capacity change (ΔC_p = -1.21 kcal mol^{-1} K^{-1}) is consistent with the hydrophobic nature of DNA/calicheamicin interaction.

Introduction

Recent studies have shown the diyne-ene-containing antitumor antibiotics calicheamicin[1] (**1**) and esperamicin[2] to be potent minor-groove DNA-cleaving agents.[3,4] For a molecule of only 1367 Da, calicheamicin shows remarkable double-strand cleavage specificity with principal sites at the 5′ penultimate pyrimidine in 5′TCCT, C*T*CT, and A*C*CT tracts and two nucleotides toward

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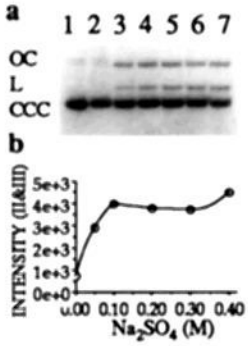
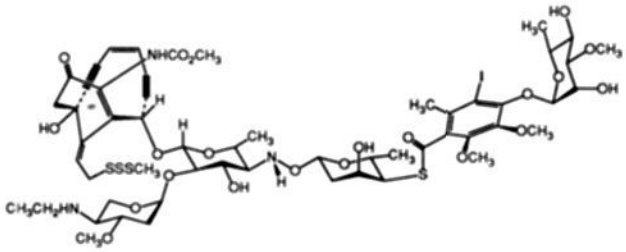


Figure 1. (a) pBR322 cutting by calicheamicin ($\gamma 1^1$) with increasing concentrations of Na_2SO_4 salt: OC, open circle; L, linear form; CCC, covalently closed circle; 5.8 nM DNA, 46 mM β-mercaptoethanol (βSH), 10 mM Tris (pH 7.5), 0.1 M NaCl, 5% EtOH, 1.46 nM $\gamma 1^1$. Lanes: 1, intact DNA; lanes 2–7, 0, 0.05, 0.1, 0.2, 0.3, and 0.4 M Na_2SO_4. Reaction at 20 °C for 10 min. (b) Quantitation of the formation of linear and open-circular plasmids by scanning densitometry (Shimadzu CS-930 at 600 nm).[12b] Arbitrary intensity is used.

the 3′ end from the complementary purine tetramer (3′*N*NAGGA). The nature of the attractive forces between these



1 calicheamicin γ_1^I

drugs and DNA is uncertain although an electrostatic component is possible, based on the cutting efficacy of calicheamicin and esperamicin, with and without the positively charged ethylamino sugar.[3,4] It is well-known, however, that hydrophobic interactions are an important factor in the binding of substrates in biological systems.[5] Thus, we speculated that hydrophobicity could be an important factor in the binding of the very lipophilic calicheamicin to the reduced dielectric regions within the minor groove of duplex DNA.[6] Although there is disagreement about the definition of hydrophobic interactions, we use it here to define the process by which nonpolar surfaces are removed from an aqueous environment. The thiobenzoate–carbohydrate tail of calicheamicin, which consists of several relatively lipophilic 6-deoxy sugars, is believed to play an important role in the binding/cleavage event with duplex DNA. Indeed, on the basis of recent NMR–NOE studies it has been suggested that the relatively lipophilic oligosaccharide moiety of calicheamicin is substantially preorganized in an extended conformation and is well suited for minor-groove binding.[7]

It has been known for some time that protein stability is affected by certain salt species primarily at high concentrations in the same order as the lyotropic or Hofmeister series.[8] Strongly hydrated anions such as SO_4^{2-} are the most stabilizing whereas weakly

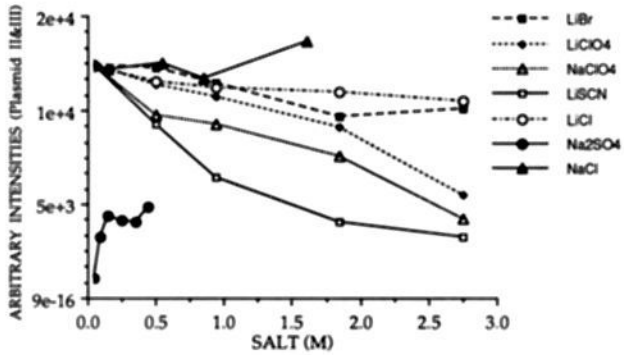


Figure 2. pBR322 cutting by $\gamma 1^1$ with increasing concentrations of salts (0–4 M): 5.8 nM DNA, 46 mM β-mercaptoethanol (βSH), 10 mM Tris (pH 7.5), 0.1 M NaCl, 5% EtOH, 14.6 nM $\gamma 1^1$. The reaction conditions and quantitation of different forms of the plasmids by scanning densitometry are the same as in Figure 1.

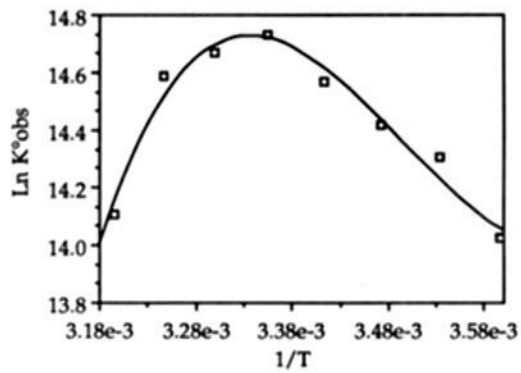


Figure 3. van't Hoff plot of ln K°_{obs} versus $1/T$ for pBR322/calicheamicin interaction (data from Table I). The curve fits a three-parameter relationship between K°_{obs} and T expected for a process in which ΔC_p is independent of temperature.[19]

hydrated anions such as ClO_4^- and CNS^- are destabilizing with Cl^- and Br^- in between. These salt effects on protein stability are generally taken as empirical evidence for hydrophobic interactions.[5e] In the first part of this paper we describe the effect of chaotropic and antichaotropic inorganic salts on the cutting efficiency of plasmid DNA by calicheamicin as an indication of the drug/DNA association.[9] In the second section we present some thermodynamic data on this interaction based on the effect of temperature. Both of these results provide strong evidence that a significant hydrophobic interaction does indeed exist in a productive calicheamicin/DNA association.

Experimental Section

The effects of the salts Na_2SO_4, NaCl, LiBr, $NaClO_4$, $LiClO_4$, and LiSCN on pBR322 DNA cutting by calicheamicin were examined. pBR322 plasmid (Boehringer Mannheim) was reacted with calicheamicin (γ_1^I) at 20 °C for 10 min (for each salt concentration, [DNA] = 5.8 nM; [β-mercaptoethanol] (βSH) = 46 mM; 10 mM Tris (pH 7.5); 5% EtOH; NaCl, 0.1M; [γ_1^I] = 1.46 nM for Na_2SO_4 (Figure 1) and 14.6 nM for other salts). NaCl (0.1M) was used in each reaction as control to minimize the ion-exchange effect of DNA.[10] The drug is chemically stable in these salt solutions, and upon treatment with thiol it was consumed within a 10-min reaction time. The formation of the final products occurred to the same extent, as shown by reversed-phase HPLC.[11] The DNA/drug ratios used in these experiments were chosen to enable the determination of the concentrations of supercoiled form I, open circular

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(11) Because of the large excess of β-mercaptoethanol in the DNA cutting reactions with each salt, more than 95% of calicheamicin was reacted within 10 min as ascertained by reversed-phase HPLC (Vydac C18 p&p 5λ; CH_3CN/H_2O, 0.1% TFA; 1 mL/min).

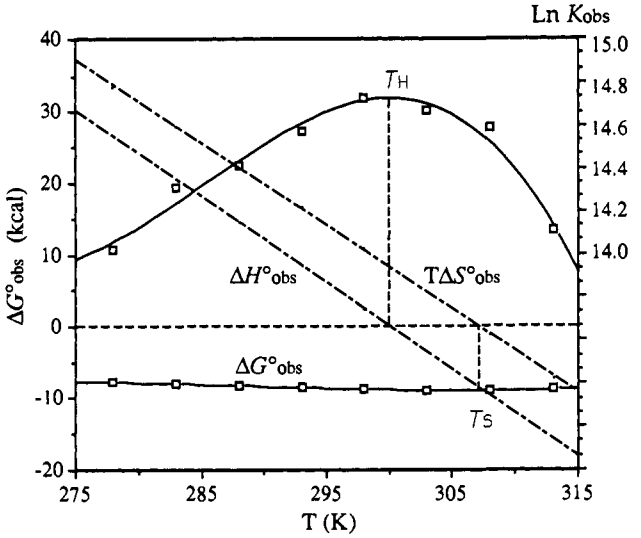

Figure 4. Thermodynamics for calicheamicin/DNA binding plotted as functions of T. ΔH°_{obs} and $T\Delta S^\circ_{obs}$ are calculated assuming ΔC_p is −1.21 kcal/T (from Table I).

form II (single-stranded nick), and linear form III (double-stranded multiple nicks) of pBR322 DNA as a function of salt concentration. Plasmids were precipitated by adding 30 μL of 3 M NaOAc (pH 5.2), 205 μL of H_2O, and 600 μL of EtOH at −20 °C to the 65-μL reaction cocktails. The suspensions were stored at −20 °C for 2 h before centrifugation at 14 000 rpm for 30 min at 4 °C. The DNA pellets were dried and dissolved in 50 μL of loading buffers. Portions of 20 μL were loaded on 1% agarose gels run at 40 V for 5 h. The gels were stained with ethidium bromide (0.5 μg/mL) for 3 h and photographed with Polaroid 55 film (negatives were treated with 5% $Na_2S_2O_3$ for 1 h). The resulting open-circular, linear, and remaining covalently closed-circular forms of the plasmids were quantitated by measuring the intensities of the DNA bands on the negative films, employing scanning densitometry (Shimadzu CS-930 at 600 nm). The spot intensities on each gel were normalized.[12]

To obtain the microscopic binding constants for TCCT, ACCT, and CTCT sequences from Scatchard plots at various temperatures (averaged from two sets of data), DNA (5.79 nM, plasmid) was reacted for 30 min with calicheamicin at concentrations of 0.366, 0.732, 1.462, 2.193, 2.924, and 3.655 nM, respectively (10 mM Tris-HCl (pH 7.5), 77 mM βSH), assuming that there are 56 binding sites per plasmid (sequence search). Excess DNA was used to achieve a single-hit kinetics. The conditions for electrophoresis and densitometry quantitation are the same as aforementioned. For the van't Hoff plot of ln K°_{obs} versus $1/T$ for pBR322/calicheamicin interaction, the curve fits a three-parameter relationship between K°_{obs} and T expected for a process in which ΔC_p is independent of temperature, as discussed by Record and co-workers.[13] T_H is the temperature at which ΔH° is 0 and T_S at which ΔS° is 0. The thermodynamic functions were derived from the following equations: $\Delta G^\circ_{obs} = -RT \ln K_{obs}$, $\Delta H^\circ_{obs} = \Delta C_p(T - T_H)$, and $\Delta S^\circ_{obs} = \Delta C_p \ln (T/T_S)$, with $\Delta C_p \approx \Delta G^\circ_{obs}/(T_S - T_H) = -1.21$ kcal T^{-1} (Figure 4).[13]

Results and Discussion

Salt Effect on Binding/Cleavage of Plasmid DNA. For the strong salting-out or antichaotropic agent Na_2SO_4, an initial concentration of drug (1.46 nM) was chosen that did not cause significant DNA strand scission (Figure 1, lane 2). Then, increasing concentrations (0.1–0.4 M) of Na_2SO_4 (Figure 1, lanes 3–7) were added to the cleavage cocktail. A concentration of 0.1 M Na_2SO_4 caused a 5-fold increase in the formation of linear and open-circular forms (Figure 1, lane 4). At higher salt concentrations, however, the formation of linear and open-circular forms leveled off (lanes 4–7). We believe this to be a result of essentially complete binding of available drug even at low Na_2SO_4 concentrations. In contrast to the results with the antichaotropic salt Na_2SO_4, the chaotropic agents such as $LiClO_4$, $NaClO_4$, and LiSCN, at increasing concentrations of 0.5–4 M, caused a dramatic decrease in cutting efficiency at a drug concentration of 14.6 nM. NaCl showed very little effect on DNA cutting, consistent with the fact that Cl^- is a neutral salt in the Hofmeister series. LiBr also reduced DNA cutting but to a lesser degree (Figure 2). $LiClO_4$ and $NaClO_4$ showed nearly identical effects despite the cation difference.

In summary of these salt effects, the hydrated surfaces of the nonpolar binding domains of the reactants are minimized on association with the release of water molecules from the drug/DNA interface. Thus, with calicheamicin, the cleavage rate acceleration observed in the presence of the strongly hydrated Na_2SO_4, a salting-out agent, is due to a decrease in the solubility of the calicheamicin by electrostriction of the solvent. As a result, the drug tends to associate more with the nonpolar regions of the minor groove of the DNA. Weakly hydrated polar salts such as $NaClO_4$, however, tend to increase the solubility of calicheamicin in water by adsorbing readily to the lipophilic surfaces of the drug.[14] This reduces the hydrophobic association between the two reactants and results in decreased binding/cleavage. Therefore, these results are the first experimental evidence that a significant part of the calicheamicin/DNA association is hydrophobic in nature, consistent with the very lipophilic carbohydrate and aglycon moieties. An interesting chemical precedent involving the effects of high ionic strength on the reactivity of DNA has recently been reported by Rokita and colleagues where they observed a significant rate acceleration of the photochemical reaction of DNA with acetone at high ionic strength.[15]

In an attempt to rule out the effect on the observed cleavage rates by possible conformational changes in the DNA at high ionic strength, the circular dichroism (CD) of pBR322 in 1–4 M LiCl and $LiClO_4$ was examined. The CD changes for both salts are essentially identical, with a strong decrease in the positive 275-nm band and essentially no change in the intensity of the negative 245-nm band over these salt concentrations. This observed change has been suggested to reflect a small conformational change within the B form.[16] Attempts to measure CD changes on titration with Na_2SO_4 were not successful due to the interference from the absorbance of the SO_4^{2-}. However, the fact that no difference in the CD profile was observed with LiCl, a salt that has no infuence on the cleavage rate, compared with $LiClO_4$, a salt that significantly reduces the rate, suggests that DNA conformational changes are probably not responsible for the differences of cleavage rate observed with the various salts used in this study. Of course it is possible that an alteration in the microenvironment at the binding site is not detected by CD.

Heat Capacity Change for the Association of Calicheamicin and DNA. In recent years it has become recognized that the most characteristic feature of hydrophobic associations is the temperature dependence of the enthalpy change (ΔH°_{obs}) in the interactions of ligand/protein,[17,18] protein folding,[19] and repressor and restriction enzyme/DNA,[13] with a large negative heat capacity change ($\Delta C_p < 0$). To further define the hydrophobic contribution to the calicheamicin/DNA association inferred above from the salt effects on the cleavage of pBR322 DNA, we have carried out a van't Hoff analysis of the plot of ln K_{obs} versus $1/T$ and calculated the ΔC_p value following the arguments of Record et al.[13] The association constant K_{obs} is calculated for sequences such as 5′TCCT, CTCT, and ACCT, which are the preferred binding sites under our reaction conditions (Table I). We assume that each drug binding is productive and causes either double-strand or single-strand cleavage, giving form III and II plasmids, respectively. Free DNA concentration is that of remaining form I

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Table I. Binding Constants and Thermodynamic Functions

T (°C)	K_{obs} ($\times 10^6$)[a]	$\Delta G°_{obs}$[b] (kcal)	$\Delta H°_{obs}$[c] (kcal)	$\Delta S°_{obs}$[c] (kcal K^{-1})
5	1.2	−7.7	26.6	0.120
10	1.6	−8.0	20.6	0.985
15	1.8	−8.2	14.5	0.077
20	2.1	−8.5	8.5	0.057
25	2.5	−8.7	2.4	0.036
30	2.3	−8.8	−3.6	0.016
35	2.2	−8.9	−9.7	−0.004
40	1.3	−8.8	−15.7	−0.023

[a] Microscopic binding constants for TCCT, ACCT, and CTCT sequences, obtained from Scatchard plots (averaged from two sets of data). [DNA] = 5.79 nM (plasmid); [drug] = 0.366, 0.732, 1.462, 2.193, 2.924, and 3.655 nM in 0.1 M NaCl/10 mM Tris-HCl, (pH 7.5) in the presence of 77 mM 2-mercaptoethanol. Reaction time was 30 min. The conditions for electrophoresis and densitometry quantitation are the same as in Figure 1.[12] [b] Calculated[13] from $\Delta G°_{obs} = -RT \ln K_{obs}$. [c] Calculated from $\Delta H°_{obs} = \Delta Cp(T - T_H)$ and $\Delta S°_{obs} = \Delta Cp \ln (T/T_S)$, respectively, with $\Delta Cp \approx \Delta G°_{obs}/(T_S - T_H) = -1.21$ kcal K^{-1}.

plasmid. The free drug concentration is obtained by substracting combined DNA forms II and III from the total amount of drug added. Excess DNA was used such that single-hit kinetics was achieved. A sequence search of pBR322 revealed that each plasmid DNA contains 64 of the aforementioned preferred binding sites.[3] Since cleavages at these sites occur to similar extents, as shown by autoradiography on sequencing gels,[3] we have assumed that they are identical and noncooperative. There are 16 sites that are less than two base pairs away from another site. Therefore, the primary binding sites should be counted as 56 per plasmid.

A recent study by Crothers et al. obtained K_{obs} values of $3.3 \times 10^7 - 1 \times 10^8$ for the binding of calicheamicin with TCCT tract by analyzing the cleavage on sequencing gels.[20] Our K_{obs} values of $\sim 10^6$ must be considered as approximate given the fact that drug binding and hydrogen abstraction may not always lead to DNA cleavage and may in fact be low. In any event, it is clear from Figure 3 that the calicheamicin/DNA interaction exhibits nonlinear van't Hoff behavior reminiscent of previously observed ligand/protein and repressor/DNA interactions.[13,18] Figure 4 summarizes the interrelationship between the thermodynamic functions for the DNA/calicheamicin association. ΔC_p was calculated to be −1.21 kcal/T, assuming that it is temperature-independent.

As for an interpretation of the negative heat capacity change, previous discussions on ligand/protein interactions have focused on the participation of water molecules. With the hydrophobic calicheamicin, the displacement of hydration water surrounding the drug along with the displacement of the spines of hydration in the minor groove of the DNA on association with calicheamicin would appear to be important contributors to the thermodynamic changes observed. Eftink and co-workers have argued that, in the case of ligand/protein interactions, any change in the state of hydration is most likely initiated by a ligand-induced change in the state of protein.[18] It may well be that a similar change in the state of the DNA is involved with the calicheamicin association. Indeed, CD experiments on titrating sonicated calf thymus DNA with the inactive aromatic calicheamicin ϵ showed a reduction in the intensity of the DNA 272-nm extremum with little or no reduction at 245-nm band.[3b] We have interpreted these results as probably due to a slight conformational change in the B-form DNA duplex brought about by the association with the drug.[16] Thus, the negative heat capacity change observed with the calicheamicin/DNA interaction might be accounted for by a combination of drug-induced changes in the hydration state of the DNA and the burial of the nonpolar surfaces of calicheamicin in a low dielectric region of the DNA minor groove. However, Sturtevant,[17] Baldwin,[19] and Record and colleagues[13] concluded from studies on ligand/protein binding, protein folding, and DNA/protein interactions that the large negative heat capacity change for these processes is best explained by the removal of nonpolar surfaces from an aqueous environment, which provides a large hydrophobic driving force for site-specific complex formation. We invoke a similar argument for the calicheamicin/DNA interaction.

The above results are consistent with recent model studies of binding in aqueous media between relatively hydrophobic ligands and guests with a hydrophobic cavity such as cyclodextrin and synthetic cyclophanes.[21] Closer to our studies are the results obtained for DNA minor-groove binding agents such as netropsin, bisbenzimide, and CC-1065, where hydrophobic interactions have been shown to play an important role in their preferred association with the AT-rich regions of the DNA receptor.[6,22] These earlier studies show that nonelectrostatic interactions provide significant binding stabilization and that the binding for netropsin and distamycin to an oligomeric and several polymeric DNA hosts is an enthalpically rather than entropically driven process between 20 and 25 °C. The calicheamicin γ_1^I binding, however, is entropically driven at these temperatures (Table I) and becomes enthalpically driven only between 30 and 40 °C.

Registry No. 1, 108212-75-5.

(20) We express our appreciation to Professors Crothers and Danishefsky for the opportunity to see a preprint of their publication. Drak, J.; Iwasawa, N.; Danishefsky, S.; Crothers, D. M. *Proc. Natl. Acad. Sci. U.S.A.*, in press.

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