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Interactions of Sulfur-Containing Acridine Ligands with DNA by ESI-MS

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Summary

The alkylating proficiency of sulfur-containing mustards may be increased by using an acridine moiety to guide the sulfur mustard to its cellular target. In this study, the interactions of a new series of sulfur-containing acridine ligands, some that also function as alkylating mustards, with DNA were evaluated by electrospray ionization mass spectrometry (ESI-MS). Relative binding affinities were estimated from the ESI-MS data based on the fraction of bound DNA for DNA/acridine mixtures. The extent of binding observed for the series of sulfur-containing acridines was similar, presumably because the intercalating acridine moiety was identical. Upon infrared multiphoton dissociation of the resulting oligonucleotide/sulfur-containing acridine complexes, ejection of the ligand was the dominant pathway for most of the complexes. However, for AS4, an acridine sulfide mustard, and AN1, an acridine nitrogen mustard, strand separation with the ligand remaining on one of the single strands was observed. At higher irradiation times, a variety of sequence ions were observed, some retaining the AS4/AN1 ligand, which was indicative of covalent binding.

Introduction

Sulfur mustards are strong alkylating agents that are known to react with many nucleophilic cell constituents, including DNA, RNA, proteins, and lipid membranes.¹ The 2-chloroethyl side chain of sulfur mustards form irreversible covalent bonds, especially with the genetic bases of DNA, ultimately causing cell death.^{1–3} Sulfur mustards are used as chemotherapeutic agents, but due to the high electrophilic property of the intermediate sulfonium ion, undesired toxicity occurs to all cells.^{1–3} We have investigated inhibiting the ability of the chloroethylthio-moiety to cyclize by oxidation of the sulfur to the corresponding sulfoxide and sulfone derivatives, with the goal of preventing direct DNA alkylation. Upon enzymatic reduction, the sulfur-oxide group may be converted to the original sulfide, allowing alkylation of DNA to occur (Scheme 1). These bioreductive antitumor agents may be used specifically to target tumors with elevated levels of sulfur-oxide reducing enzymes.

In order to guide a sulfur mustard to its cellular target, we have designed a new generation of sulfur mustards that contain an acridine moiety, which are known to bind DNA through intercalation (Figure 1).^{4–8} Numerous intercalators have exhibited anti-cancer properties,^{9–10} with actinomycin-D, daunomycin and adriamycin being examples of these agents. Binding is achieved through intercalation of the tri-cyclic ring between adjacent base pairs in the DNA duplex.^{11–12} Van der Waals forces as well as electrostatic interactions with the phosphates of the DNA backbone secure the acridine.

We have previously investigated the DNA binding of a series of intercalating acridine compounds having sulfide or sulfur-oxide mustards through various solution-phase assays.¹³ Compounds containing a chloroethyl sulfide moiety alkylated DNA much more effectively than compounds that contained the chloroethyl sulfoxide or chloroethyl sulfone group. This result is consistent with our hypothesis that oxidation of the sulfide will inhibit the alkylating activity of the mustard group. In vitro MTT assays indicated that compounds containing a chloroethyl sulfide or chloroethyl sulfone group showed promising cytotoxic activity in human cancer cell lines. Surprisingly, compounds containing a chloroethyl sulfoxide group showed little or no cytotoxic activity. The chloroethyl sulfoxide should be easier to reduce to a sulfide than the sulfone group, indicating that other mechanisms may be involved in the cytotoxic activity of these types of compounds.

In order to understand the relationship between this new series of acridine ligands and their interaction with DNA, we have extended our study of these compounds by electrospray ionization mass spectrometry (ESI-MS). ESI-MS has emerged as a useful technique for the analysis of duplex DNA and associated DNA-binding agents for ligands that bind both covalently or noncovalently.^{14–48} Due to its low sample consumption and fast analysis time, ESI-MS provides many advantages as a screening technique for DNA/drug complexes. During the ESI process, both covalent and noncovalent complexes in solution can be transferred to the gas phase with conservation of many of the intermolecular interactions formed in the solution environment. The successful transfer of these complexes to the gas phase allows information about binding stoichiometry, sequence selectivity, and relative binding affinity to be determined.^{14–48} Several groups, including our own,^{35–48} have focused on developing ESI-MS based techniques to correlate the binding of duplex interactive ligands observed in the gas-phase to known solution behavior with the ultimate goal of developing ESI-MS as a screening tool for DNA/ligand complexes.

Results and Discussion

To characterize the DNA binding properties of the new acridine compounds, a multi-faceted approach was followed. ESI-MS was used to evaluate the stoichiometries of the duplex/sulfur-containing acridine complexes and assess the sequence selectivities and relative binding affinities of the acridine ligands. Two activation techniques, IRMPD and CID, were used to map the dissociation patterns of the complexes. UV-Vis absorption spectroscopy was used to determine the increase in duplex stability upon ligand addition by mapping the change in melting temperature of a DNA duplex upon complexation with a sulfur-containing acridine ligand.

The mass-to-charge ratios of the ions observed in the ESI mass spectra of each DNA/acridine incubate give basic information about the reaction state of the mustard moiety. Each sulfur-containing chloroethyl acridine (AS4, AS5, and AS6) has the potential to form both non-covalent complexes and covalent adducts with DNA, the latter of which competes with hydrolysis. After intercalation, if the mustard moiety remains unreacted, then the m/z value of the noncovalent DNA/acridine ligand complex should contain the entire mass of the acridine ligand. In contrast, upon covalent adduction to DNA, the resulting product should be shifted in mass corresponding to loss of the chlorine atom. Moreover, if the chloroethyl moiety undergoes hydrolysis, a mass shift of the DNA complex that corresponds to loss of chlorine and the addition of a hydroxyl group will be observed.

Binding stoichiometries

The ESI-mass spectra obtained for solutions containing 10 μM of one duplex or 10 μM of one oligonucleotide and 50 μM of one of the sulfur-containing acridine ligands in a 50 mM ammonium acetate 3:1 water/methanol solution exhibited complexes having a variety of

binding stoichiometries. The mass spectrum of each duplex alone is dominated by ions of the 5- and 6- charge states of the intact duplex, in addition to some unannealed single strand species of low abundance in the 3- charge state (spectra not shown). For the mass spectrum of each oligonucleotide alone, ions corresponding to the single strand in the 2- and 3- charge states are the only dominant species.

AS1, AS2, and AS3 are structurally similar but do not contain a chloroethyl moiety, and therefore have no potential to alkylate DNA. A typical ESI-mass spectrum of the solution containing AS1 with duplex 1 is shown in Figure 2a. The duplex/AS1 complexes possess binding stoichiometries ranging from 1:1 to 1:4. The most abundant complex has a 1:2 duplex:AS1 stoichiometry, with the abundance of each duplex/AS1 complex decreasing as the associated number of ligands increases. The overall extent of DNA/AS1 binding is similar to that observed for the other acridine sulfur alcohols and acridine sulfur chloroethyl ligands, suggesting that the acridine portion of the ligand, regardless of the absence or presence of the chlorine atom as part of the mustard moiety, is responsible for the substantial DNA-binding affinity of these ligands. In fact, the presence of the hydroxyl group in place of the mustard functionality seems to increase the non-covalent binding affinity.

A typical ESI-mass spectrum of the solution containing AS1 with single strand 2 is shown in Figure 2b, and the mass spectra acquired for the other acridine alcohol and acridine chloroethyl ligands and single strand combinations revealed similar abundances and types of complexes. The single strand/AS1 complexes possess binding stoichiometries ranging from 1:1 to 1:3 in the 3- charge state and 1:1 to 1:2 in the 2- charge state. In addition, the mass spectrum shows the emergence of new products corresponding to two single stranded DNA species bound to the sulfide acridine alcohol. These “two strand”/AS1 complexes possess binding stoichiometries ranging from 2:1 to 2:4, with the abundance of each complex varying non-uniformly as the number of associated ligands increases. Each acridine ligand, AS1, AS2, AS3, AS4, AS5, AS6, and AN1, formed these unique “two strand”/acridine complexes. Because the single strands used in this study are either self-complementary or are near self-complementary, we attempted to form the “two strand” complexes alone and in the presence of other common intercalators and minor groove binders. Single strand 1 formed a small amount of duplex without the presence of a ligand (spectrum not shown). However, single strand 2 and 3 did not form any “two strand” complex unless a sulfur-containing acridine ligand was present in solution.

The single strand complexes formed with AS2, AS3, AS4, AS5, AS6 and AN1 exhibit 1:1 and 1:2 binding stoichiometries, and 2:1, 2:2, 2:3, and 2:4 stoichiometries are observed for the corresponding [ss+ASX+ss] two strand complexes. Based on IRMPD data presented later, there is no evidence to support that these two stranded complexes adopt conventional base-paired duplex structures. The acridine ligand more likely engages in electrostatic/intercalative interactions with both strands, forming a [ss+acridine+ss]-type complex. This conclusion is supported by the dissociation patterns of these complexes: release of an intact duplex is not a dominant fragmentation pathway.

Inspection of a typical mass spectrum acquired for the solution containing AS4 with either duplex DNA or single stranded DNA reveals DNA/AS4 complexes that incorporate a mass shift corresponding to the loss of the chlorine atom from the chloroethyl acridine, suggesting near complete covalent adduction to DNA, presumably via the type of alkylation process shown in Scheme 1. The duplex/AS4 complexes possess binding stoichiometries ranging from 1:1 to 1:2, with the abundance of each complex decreasing as the number of associated ligands increases.

The mass spectrum of AS5 with either duplex or single stranded DNA primarily shows products that incorporate the entire acridine moiety with no mass shift, i.e. (acridine)-NH(CH₂)₃SOHCH₂CH₂Cl, which suggests the chloroethyl unit remains unreactive. The duplex/AS5 complexes possess binding stoichiometries ranging from 1:1 to 1:2, with the abundance of each complex decreasing as the number of associated ligands increases.

The ESI-mass spectrum in Figure 3 was acquired for a solution containing AS6 with duplex 1. The duplex/AS6 complexes possess binding stoichiometries ranging from 1:1 to 1:2, with the abundance of each complex decreasing as the number of associated ligands increases. Two mass-shifted products, in addition to the original complex, are present, indicating a mixture of these three species: [(acridine)-NH(CH₂)₃SO₂CH₂CH₂-DNA], [(acridine)-NH(CH₂)₃SO₂CH₂CH₂OH + DNA], and [(acridine)-NH(CH₂)₃SO₂CH₂CH₂Cl + DNA]. Based on IRMPD results shown later, however, we hypothesize that the presumed alkylated DNA product is in fact one in which the mustard moiety has converted to a vinyl sulfone, resulting in a noncovalent DNA complex of the form [(acridine)-NH(CH₂)₃SO₂CH=CH₂ + DNA].

Interestingly, the trend in formation of the products involving the loss of the chlorine from the chloroethyl acridine (i.e. the mass-shifted ones via alkylation of DNA or conversion of the chloroethyl moiety to a vinyl sulfone) versus the formation of complexes containing unreacted or hydrolyzed chloroethyl moieties (i.e. simple association of the acridine with DNA) parallels findings from our previous MTT assays in which compounds containing a chloroethyl sulfide (AS4) or chloroethyl sulfone (AS6) showed good cytotoxic activity in human cancer cell lines and, surprisingly, compounds containing a chloroethyl sulfoxide (AS5) showed little or no cytotoxic activity.¹³ The chloroethyl sulfoxide (AS5) should be more easily reduced to a sulfide (AS4) than the sulfone group (AS6), indicating that other factors may be involved in the cytotoxic activity of these types of compounds. The ESI-MS results suggest that the mechanistic details that enhance the reactivity of AS6 over AS5 do not necessarily require a cellular environment, because this interesting feature occurs both within cells and solutions containing only DNA and ligand.

The complexation of AN1 was also evaluated because of its structural similarity to the acridine chloroethyl sulfur compounds, being an acridine derivative, but containing a nitrogen mustard alkylating moiety. In order to retain the same spacer distance between the central sulfur/nitrogen atom of the mustard moiety and the nitrogen atom bridging the acridine, the number of carbons in the linker was increased from three to four. The complexation of AN1 with each of the duplexes and single strands was evaluated by ESI-MS, and the complexes exhibit binding stoichiometries similar to those observed for AS2, AS3, AS4, AS5, and AS6 with 1:1 and 1:2 complexes observed for duplex DNA, 1:1 and 1:2 complexes detected for single stranded DNA, and 2:1, 2:2, 2:3, and 2:4 stoichiometries observed for the two strand complexes. The mass spectra acquired for solutions containing AN1 with either duplex or single stranded DNA primarily display DNA/AN1 complexes that reflect a mass shift corresponding to the loss of the chlorine atom from the acridine nitrogen mustard, suggesting near complete covalent adduction of AN1 to DNA, similar to the covalent binding properties of AS4. The similar overall binding of AN1 relative to the chloroethyl sulfur compounds supports the conclusion that the acridine portion of the ligands, and not the central sulfur/nitrogen atom of the mustard moiety, is responsible for the substantial DNA-binding affinities of these ligands.

Relative binding affinities

Comparison of the relative binding affinities of the sulfur-containing acridine ligands for duplex and single stranded DNA as assessed by ESI-MS (for the type of products shown in Figures 3 and 4) is summarized in bar graph form in Figure 4a and 4b, respectively. The extent of DNA binding, expressed as a “fraction bound” value that equates with the relative binding affinity of each acridine ligand, was determined based on the following equation:⁴¹

$$\text{Fraction of bound DNA} = \frac{A_{(1:1)} + A_{(1:2)} + A_{(1:3)} + \dots}{A_{(\text{DNA})} + A_{(1:1)} + A_{(1:2)} + A_{(1:3)} + \dots} \times 100\%$$

where A_{DNA} is the peak area of the free duplex or single strand species and $A_{(1:n)}$ are the peak areas of all DNA/acridine complexes in the ESI-mass spectra. All calculated fractions of bound DNA assume the peak areas of the free and bound DNA ions are proportional to their relative concentrations in solution. The fraction of bound DNA is a relative comparison of the extent of ligand binding, in contrast to reporting absolute values that could be affected significantly by ESI response factors (i.e. ESI spray efficiencies of different DNA species). Higher values of the fraction bound parameter suggest a greater DNA/sulfur-containing acridine ligand binding affinity. Three replicates were undertaken for each solution.

Based on the fraction bound values shown in Figure 4, AS1 consistently shows the greatest relative DNA binding affinity, both for duplexes and single strands. AS2, AS3, AS4 and AS6 have similar and slightly lower binding affinities, and AS5 and AN1 exhibit even lower binding affinities. The differences in relative DNA binding affinities among all the sulfur-containing acridines are rather modest. The acridine portion of each ligand is identical, and this is expected to be the dominant factor which influences non-covalent binding to DNA.

To assess the sequence selectivity of the sulfur-containing acridines, the relative extent of binding to four different duplexes with varying A-T base pair composition was evaluated (see Figure 4a). The second duplex has the same base composition as the first, but its sequence follows the pattern 5' – GNC – 3'. Recent studies have shown nitrogen mustards prefer 5' – GNC – 3' sequences.^{50–53} Duplex 3 is a mixed base sequence containing a moderate number of all bases. Duplex 4 is very AT rich, with only two CG base pairs contained in the duplex. The ESI-MS results in Figure 4a indicate that the sulfur-containing acridine ligands tend to prefer a mixed base sequence (duplex 3) more than the GC-rich sequences (duplex 1 and 2) or the AT-rich sequence (duplex 4). However, the differences in relative binding are not large, indicating that these acridine ligands exhibit little selectivity between the duplexes in the study. Interestingly, sequence selectivity was much more apparent for the solutions in which the acridines were incubated with single stranded DNA (Figure 4b). Relative binding was consistently higher for the GC-rich sequence (ss1), followed by the mixed base sequence (ss2), with the lowest binding for the AT-rich, guanine-free sequence (ss3). In addition, covalent adduction is observed with the AT-rich single strand (ss3), indicating that the mustard can also bind to adenines.

IRMPD of sulfur-containing acridine ligand/duplex DNA complexes

In the present study, we exploit the benefits of IRMPD to characterize the DNA/ligand complexes along with acquisition of CID mass spectra for comparative purposes. IRMPD has proven useful as an alternative approach to traditional CID for gaining sequence information of DNA molecules as well as sites of ligand adduction.^{36,43,45} IRMPD offers two main benefits over CID in quadrupole ion traps. The non-resonant process of ion activation by IR absorption results in rapid conversion of the uninformative base loss ions, ones that often dominate CID mass spectra acquired in quadrupole ion traps, into *a-B* and *w* sequence ions without the need for sequential MS/MS stages. Secondly, during IRMPD, ion activation is independent of the *rf* trapping voltage, giving a broader *m/z* range that extends to lower *m/z* values and thus allowing detection of single nucleobase species and their modified counterparts. This important lower *m/z* range is lost due to constraints on the *rf* trapping voltage during collision activation. CID of noncovalent duplex DNA/intercalator complexes has been reported previously.^{30,32,33,40,46} Duplex/intercalator complexes may dissociate by loss of the intercalating ligand, leaving the intact duplex.^{33,40,46} Depending on charge state, intercalators that bind more

strongly to DNA promote strand separation, with the intercalating ligand bound to one of the two strands.^{33,40} Ligands that bind covalently to DNA often show significantly different MS/MS patterns.^{54–58} Depending on charge state, covalent DNA-ligand complexes may dissociate via strand separation and upon sequential CID steps, dissociation of the modified single strand results in formation of *a-B* and *w* ions, with the covalent modification being retained on specific diagnostic sequence ions. Another common fragmentation pathway that is observed when the ligand is covalently bound to a nucleobase is loss of the modified base. Single strand DNA that has been covalently modified by a ligand dissociates by similar routes.

In this study, each DNA/acridine complex was isolated and subjected to IRMPD and CID. The complexes containing AS1, the acridine sulfide alcohol which has the ability to associate only non-covalently with DNA by intercalation, dissociated via loss of AS1 from the intact duplex upon MS/MS for both the 5- and 6- charge states (Supplemental Figure 1a, 1c). This IRMPD pattern supports a non-covalent binding mode and is consistent with our previous results that indicate AS1 has no alkylation activity.

AS5, the chloroethyl acridine sulfoxide, formed complexes with duplex DNA that were consistent with addition of the entire ligand. IRMPD of these complexes resulted in loss of the ligand, leaving the intact duplex, supporting a non-covalent interaction (Supplemental Figure 1b and 1d). Partial oxidation of the central sulfide to a sulfoxide appears to completely inhibit covalent adduction to DNA. Longer IR irradiation results in separation of the duplex strands (6- charge state) or base loss (5- charge state), but never with the retention of AS5.

AS4, the acridine sulfide mustard, formed complexes with duplex DNA that were consistent with alkylation of the DNA. IRMPD of these complexes in the 6- charge state resulted in strand separation, with the AS4 ligand bound to either strand (Figure 5a). In addition, loss of AS4 with a guanine base is observed, as well as loss of a guanine base from the duplex with retention of AS4. Interestingly, two products are detected that correspond to each of the single strands retaining both the AS4 moiety *as well as* a single guanine base, i.e. [ss+AS2+GH]. This product offers evidence that the AS4 ligand covalently binds to guanine, thus allowing the guanine from one strand to remain associated with the second strand upon the IR-activated strand separation. The presence of this type of product ion also suggests that when a guanine and the AS4 moiety are ejected from a single strand during IRMPD, they are likely leaving together as a bound unit. IRMPD of the 5- charge state of the same alkylated complex resulted predominantly in loss of a guanine and AS4 from the duplex. At longer irradiation times, secondary fragmentation of the DNA produces *a-B* and *w* ions, some of which retain the AS4 ligand (Figure 5b).

AS6, the chloroethyl acridine sulfone, formed several complexes with duplex DNA including ones that were consistent with association of the entire ligand, one that also entailed loss of the chlorine atom, and one that involved loss of the chlorine with an addition of a hydroxyl group. Each of these products was subjected individually to IRMPD. Supplemental Figure 2a-c shows the fragmentation pattern of each duplex 2/AS6 complex, all of which are similar. Each complex primarily dissociated by simple loss of the AS6 moiety, thus suggesting that all three complexes are non-covalent ones (Figure 3). We speculate that instead of covalent adduction to guanine, loss of the chlorine from the chloroethyl acridine sulfone initiates the formation of an acridine vinyl sulfone (Supplemental Figure 2d), which intercalates into DNA but does not alkylate DNA like AS4.

AN1, the acridine nitrogen mustard, formed complexes with duplex DNA that were consistent with alkylation of the DNA, based on its *m/z* value. IRMPD of these complexes in the 6- charge state resulted in strand separation, with the AN1 ligand remaining bound to one of the two strands. Other pathways include the concurrent loss of the AN1 moiety with the loss of a

guanine base, or loss of a guanine base from the duplex with the AN1 ligand retained. IRMPD of the 5- charge state resulted predominantly in loss of a guanine base and AN1 from the duplex (spectra not shown). These IRMPD spectra confirm that the AN1/duplex complexes are covalent ones.

To evaluate the potential of IRMPD to pinpoint the exact location of the AS4 adduction sites, several IRMPD spectra were collected for each (duplex + AS4) complex as the irradiation time was increased to enhance secondary fragmentation and thus promote formation of sequence ions. A summary of results obtained by IRMPD is shown in Scheme 2 in which the slash marks along the DNA sequences represent the detection of complementary *a* – *B* and *w* product ions which are the conventional diagnostic sequence ions observed for DNA. High sequence coverage of the duplexes is obtained by IRMPD. Moreover, the slash marks with arrows represent the detection of sequence ions that retain the AS4 ligand. In most cases, this latter series of fragment ions can be used to identify the specific alkylation sites, typically guanines. Guanines that are shown in bold font in Scheme 2 are the sites of AS4 adduction. Likely sites of AS4 adduction are determined if both series of ions (*w* and *a*-*B*) retain the ligand surrounding the guanine of interest. Several guanines in each strand of duplexes 1, 2, and 3 are likely sites of AS4 adduction. All duplexes exhibit several sites of AS4 adduction. Interestingly, most adducted guanines appear in complementary 5'-CG steps. In duplex 4 (the AT-rich strand) the terminal adenines also appear to react with AS4, although a single specific adenine reaction site cannot be pinpointed from the IRMPD data.

IRMPD of sulfur-containing acridine ligand/single strand DNA complexes

Based on assessment of the *m/z* values of the DNA/ligand complexes in the ESI-mass spectra and the IRMPD mass spectra, the mode of binding of each sulfur-containing acridine appears to be consistent for both duplex and single strand DNA. AS1, AS2, AS3, AS5, and AS6 form noncovalent complexes with single stranded DNA, whereas AS4 and AN1 both alkylate DNA. IRMPD of the single strand complexes with AS1, AS2, AS3, AS5, and AS6 resulted in loss of the neutral ligand from the intact DNA. IRMPD of the single strand/AS4 complex, [ss2 + (AS4 – Cl)]³⁻, (Figure 6a) resulted predominantly in loss of the AS4 moiety with a guanine base, or loss of adenine alone. Another interesting fragment ion formed from the [ss2 + AS4]³⁻ precursor is [G + (AS4 – Cl)]⁻, a product in which the AS4 ligand must be directly attached to a guanine base, which further confirms the ability of AS4 to alkylate the guanine base. In addition, one diagnostic fragment ion that retains the AS4 ligand is observed: [a₇ + AS4]²⁻. This latter fragment ion suggests that the AS4 ligand has alkylated the guanine at the a₇ position because loss of guanine at the 3' end is not observed, nor does the complementary w₆²⁻ ion ever retain the AS4 moiety.

Figure 6b displays the fragmentation pattern of the unusual [ss2 + (AS4 – Cl) + ss2] complex. Several fragments are observed including the intact single strand and the single strand with the (AS4 – Cl) ligand attached, similar to a typical strand separation process often observed upon IRMPD of duplexes. Loss of a guanine in conjunction with the (AS4 – Cl) ligand also occurs. Another interesting fragment corresponds to the single strand retaining the (AS4 – Cl) adduct *as well as* a single guanine base, resulting in a product of the type [ss2 + (AS4 – Cl) + GH]. This same type of product was also observed upon IRMPD of the duplex/AS4 complexes.

Melting point studies of sulfur-containing acridine ligand/DNA complexes

The change in melting temperature of duplex 3 upon addition of each of the sulfur-containing acridines is shown in Supplemental Table 1. The free duplex has a melting temperature of 43.2 ± 0.9 °C. Intercalation of a ligand may increase the thermodynamic stability of duplex, thereby increasing the observed melting temperature.⁵⁹ DNA ligands that bind solely through covalent adduction do not shift the melting temperature of DNA,⁶⁰ so the ability of AS4 and AN1 to

alkylate DNA should not influence the melting point values. AS1, AS2, AS3, AS4, AS5, AS6 and AN1 all increase the melting temperature of DNA by between 6.0 to 7.9 °C, suggesting that each acridine moiety has similar noncovalent binding properties, most likely through intercalation. Because AS1, AS2, AS3, AS4, AS5, AS6 and AN1 have identical acridine moieties, it is expected that the melting temperatures of the resulting DNA complexes should be similar, as observed.

Experimental

Chemicals

Detailed synthetic procedures for the preparation of the sulfur containing acridines will be discussed in a separate forthcoming publication. Stock solutions of the sulfur-containing acridines were prepared in acetonitrile and stored at room temperature in the dark.

The nine-mer oligodeoxynucleotides, ss1 = 5'-CGCGCGCGC-3', ss2 = 5'-ACGTACGTT-3', and ss3 = 5'-ATATAAATA-3', were custom synthesized on an in-house DNA synthesizer on the 1.0 uM scale. HPLC-purified ammonium salts of the following oligodeoxynucleotides were purchased from Integrated DNA Technologies (Coralville, IA) on the 1.0 uM scale and used without further purification: 5'-GCGCGGAACCGCGC-3', 5'-GCGCGGTTCCGCGC-3', 5'-GGCGTCGGCGTCGC-3', 5'-GCGACGCCGACGCC-3', 5'-ACGTTACGTTACGC-3', 5'-GCGTAACGTAACGT-3', 5'-ATAAAACGAAATA-3' and 5'-TATTTTCGTTTAT-3'. DNA single strand concentrations were determined spectrophotometrically by Beer's Law using the extinction coefficients provided by the manufacturer. Annealing was performed by preparing stock solutions containing 2 mM of each complementary ODN in 100 mM ammonium acetate. The solutions were heated to 90°C for 10 min and slowly cooled to room temperature overnight. The following duplexes were created: duplex 1 = 5'-GCGCGGAACCGCGC-3'/5'-GCGCGGTTCCGCGC-3', duplex 2 = 5'-GGCGTCGGCGTCGC-3'/5'-GCGACGCCGACGCC-3', duplex 3 = 5'-ACGTTACGTTACGC-3'/5'-GCGTAACGTAACGT-3', and duplex 4 = 5'-ATAAAACGAAATA-3'/5'-TATTTTCGTTTAT-3'. For each duplex created above, the oligonucleotide listed first is defined as "ssa", and the oligonucleotide listed second is defined as "ssb". A summary of the duplex and single strands studied is shown in Table 1.

DNA/acridine ligand complexes were formed by incubating 100 µM DNA with 500 µM acridine ligand in 10 mM ammonium acetate at 37°C for 3 hours. For the binding studies involving ss DNA, one acridine ligand was incubated simultaneously with all three single stranded oligonucleotides in order to eliminate error in relative binding determinations. For the studies involving duplexes, each acridine ligand was incubated individually with each duplex, and the resulting incubates were analyzed back-to-back. For ESI-MS analysis, the incubate was diluted to 10 µM of DNA and 50 µM of sulfur-containing acridine ligand by preparing in 50 mM ammonium acetate solution containing 25% methanol at a nominal pH of 6.5. For UV-Vis analysis, the incubate was diluted to 2.5 µM DNA, 12.5 µM sulfur-containing acridine ligand, and 12.5 mM ammonium acetate at a nominal pH of 6.5.

Mass spectrometry

Electrospray ionization mass spectra were collected on a ThermoFinnigan LCQ Duo mass spectrometer (San Jose, CA). A Harvard syringe pump (Holliston, MA) set at a flow rate of 3 µL/min was used to directly infuse the sample solutions into the mass spectrometer. The ESI source was operated in the negative ion mode with an electrospray voltage of 3.5 kV and a heated capillary temperature of 90 °C. To assist in desolvation, nitrogen sheath and auxiliary gas were applied at 40 and 20 arbitrary units, respectively. Spectra were acquired by summing 20 scans.

IRMPD was carried out on a ThermoFinnigan LCQ Deca XP (San Jose, CA) modified for a model 48-5 Synrad 50 W CO₂ continuous wave laser (Mukilteo, WA).⁴⁹ The laser was triggered during the activation portion of the scan function for photodissociation using a q_z value of 0.10 for IRMPD experiments. This q_z value resulted in a broader m/z storage range (i.e. extension of lower m/z range) than for CID experiments. The typical irradiation times for IRMPD were 1.0 – 3.0 ms at 50 W for DNA/acridine complexes unless otherwise stated.

In the collisionally induced dissociation (CID) experiments, activation voltages were applied at a level required to reduce the isolated precursor ion to ~10–20% of its original abundance. The default activation time of 30 ms was used in all CID experiments with a q_z value of 0.25.

UV-Vis spectroscopy

Absorbance spectra were recorded on a Cary 100 Bio UV-Visible spectrophotometer. Thermal denaturation was accomplished by heating quartz cell with a Cary Temperature Controller at 1°C/min. Absorbance was monitored at 260 nm and read at intervals of 0.06°C. An increase in temperature denatures the duplex into single strands, and as melting of the strands occurs, hyperchromism is measured by the UV spectrophotometer.^{19,59} The melting temperature T_m was obtained by taking the maximum of the derivative. The melting temperature represents the transition midpoint. The change in the melting temperature (ΔT_m) was calculated by subtracting the T_m of DNA without ligand from that with ligand. The melting temperature of the uncomplexed DNA was 43.2 ± 0.9 °C.

Conclusion

Electrospray ionization mass spectrometric analysis of selected duplexes and single strands with the acridine ligands indicated they readily formed complexes of various stoichiometries. The use of ESI-MS allowed rapid and efficient comparison of the relative binding affinities of the sulfur-containing acridine ligands with minimal sample consumption. The relative binding affinities of the sulfur-containing acridines varied with sequence, with AS1 showing the greatest relative binding affinity. Upon infrared multiphoton dissociation of the oligonucleotide/acridine complexes, ejection of the ligand was the dominant pathway for most of the complexes. However, for AS4, the acridine sulfide mustard, and AN1, the acridine nitrogen mustard, strand separation with the ligand remaining on one of the single strands was observed. At even higher irradiation times, a variety of sequence ions were observed, some retaining the AS4 or AN1 ligand which was further indicative of alkylation. Alkylation most often occurred at guanine bases, although low level adduction to adenine was also observed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Bignold LP. Anticancer Research 2006;26:1327. [PubMed: 16619541]
2. Peck RM, O'Connell AP, Creech HJ. Journal of Medicinal Chemistry 1966;9:217. [PubMed: 5911801]
3. Ghanei M, Harandi AA. Inhalation Toxicology 2007;19:451. [PubMed: 17365048]

4. Ferguson LR, Denny WA. *Mutation Research* 2007;623:14. [PubMed: 17498749]
5. Belmont P, Bosson J, Godet T, Tiano M. *Anti-Cancer Agents in Medicinal Chemistry* 2007;7:139. [PubMed: 17348825]
6. Martinez R, Chacon-Garcia L. *Current Medicinal Chemistry* 2005;12:127. [PubMed: 15638732]
7. Denny WA. *Current Medicinal Chemistry* 2002;9:1655. [PubMed: 12171548]
8. Demeunynck M, Charmantray F, Martelli A. *Current Pharmaceutical Design* 2001;7:1703. [PubMed: 11562307]
9. Blake A, Peacocke AR. *Biopolymers* 1968;6:1225. [PubMed: 5669464]
10. Belmont P, Bosson J, Godet T, Tiano M. *Anti-Cancer Agents in Medicinal Chemistry* 2007;7:139. [PubMed: 17348825]
11. Sakore TD, Jain SC, Tsai C, Sobell HM. *Proc Natl Acad Sci USA* 1977;74:188. [PubMed: 264674]
12. Waring MJ. *J Mol Biol* 1970;54:247. [PubMed: 5500444]
13. Begleiter, A.; Raza, N.; Pickering, CM.; Leith, MK.; Guzic, LJ.; Pritchard, CN.; Bruns, KA.; Guzic, FS, Jr. *America Association for Cancer Research Meeting*; 2006.
14. Gale DC, Smith RD. *J Am Soc Mass Spectrom* 1995;6:1154.
15. Triolo A, Arcamone FM, Raffaelli A, Salvadori P. *J Mass Spectrom* 1997;32:1186. [PubMed: 9373959]
16. Kapur A, Beck JL, Sheil MM. *Rapid Commun Mass Spectrom* 1999;13:2489. [PubMed: 10589098]
17. Greig ML, Robinson JM. *J Biomol Screen* 2000;5:441. [PubMed: 11598462]
18. Wan KX, Shibue T, Gross ML. *J Am Chem Soc* 2000;122:300.
19. Gabelica V, Rosu F, Houssier C, De Pauw E. *Rapid Commun Mass Spectrom* 2000;14:464. [PubMed: 10717657]
20. Iannitti-Tito P, Weimann A, Wickham G, Sheil MM. *Analyst* 2000;125:627. [PubMed: 10892019]
21. Gupta KA, Beck JL, Sheil MM. *Rapid Commun Mass Spectrom* 2001;15:2472. [PubMed: 11746919]
22. Rosu F, Gabelica V, Houssier C, De Pauw E. *Nucleic Acids Res* 2002;30:82.
23. Carrasco C, Rosu F, Gabelica V, Houssier C, De Pauw E, Garbay-Jaureguiberry C, Roques B, Wilson WD, Chaires JB, Waring MJ, Bailly C. *Chem Biochem* 2002;3:1241.
24. Colgrave ML, Beck JL, Sheil MM, Searle MS. *Chem Commun* 2002;6:556.
25. Guittat L, Alberti P, Rosu F, Van Miert S, Thetiot E, Pieters L, Gabelica V, De Pauw E, Ottaviani A, Riou JF, Mergny JL. *Biochimie* 2005;85:547.
26. Gabelica V, Galic N, Rosu F, Houssier C, De Pauw E. *J Mass Spectrom* 2003;38:491. [PubMed: 12794869]
27. Colgrave ML, Iannitti-Tito P, Wickham G, Sheil MM. *Aust J Chem* 2003;56:401.
28. Rosu F, De Pauw E, Guittat L, Alberti P, Lacroix L, Mailliet P, Riou JF, Mergny JL. *Biochemistry* 2003;42:10361. [PubMed: 12950163]
29. Beck JL, Gupta R, Urathamakul T, Williamson NL, Sheil MM, Aldrich-Wright JR, Ralph SF. *Chem Commun* 2003;5:626.
30. Gabelica V, De Pauw E. *J Am Soc Mass Spectrom* 2001;13:91. [PubMed: 11777205]
31. Gabelica V, De Pauw E, Rosu F. *J Mass Spectrom* 1999;34:1328. [PubMed: 10587629]
32. Wan KX, Gross ML, Shibue T. *J Am Soc Mass Spectrom* 2000;11:450. [PubMed: 10790849]
33. Rosu F, Pirotte S, De Pauw E, Gabelica V. *Int J Mass Spectrom* 2006;253:156.
34. Reyzer ML, Brodbelt JS, Kerwin SM, Kumar D. *Nucleic Acids Research* 2001;29:103.
35. David W, Kerwin SM, Kern J, Brodbelt JS. *Anal Chem* 2002;74:2029. [PubMed: 12033303]
36. Keller KM, Brodbelt JS. *Analytical Biochemistry* 2004;326(2):200. [PubMed: 15003561]
37. Oehlers L, Mazzitelli C, Rodriguez M, Brodbelt JS, Kerwin S. *J Am Soc Mass Spectrom* 2004;15:1593. [PubMed: 15519226]
38. Keller KM, Brodbelt JS. *J Amer Soc Mass Spectrom* 2005;16:28. [PubMed: 15653361]
39. Keller KM, Zhang J, Breeden MM, Ellington AD, Brodbelt JS. *J Mass Spectrom* 2005;40:1362. [PubMed: 16220501]
40. Keller KM, Zhang J, Oehlers L, Brodbelt JS. *J Mass Spectrom* 2005;49:1327. [PubMed: 16217837]

41. Mazzitelli CL, Kern JT, Rodriguez M, Brodbelt JS, Kerwin S. J Am Soc Mass Spectrom 2006;17:593. [PubMed: 16503153]
42. Sherman CL, Pierce SE, Brodbelt JS, Tuesuwan B, Kerwin SM. J Am Soc Mass Spectrom 2006;17:1342. [PubMed: 16872840]
43. Wilson JJ, Brodbelt JS. Analytical Chemistry 2007;79:2067. [PubMed: 17249688]
44. Mazzitelli CL, Chu Y, Reczek JJ, Iverson BL, Brodbelt JS. J Am Soc Mass Spectrom 2007;18:311. [PubMed: 17098442]
45. Mazzitelli CL, Brodbelt JS. Analytical Chemistry 2007;79:4636. [PubMed: 17508717]
46. Smith SI, Guziec LJ, Guziec FS Jr, Hasinoff BB, Brodbelt JS. Journal of Mass Spectrometry 2007;42:681. [PubMed: 17405184]
47. Mazzitelli CL, Wang J, Smith SI, Brodbelt JS. J Am Soc Mass Spectrom 2007;18:1760. [PubMed: 17719795]
48. Mazzitelli CL, Rodriguez M, Kerwin SM, Brodbelt JS. J Am Soc Mass Spectrom 2008;19:209. [PubMed: 17583529]
49. Wilson JJ, Brodbelt JS. Analytical Chemistry 2006;78:6855. [PubMed: 17007506]
50. Brookes P, Lawley PD. Biochemical Journal 1961;80:496. [PubMed: 16748923]
51. Rink SM, Solomon MS, Taylor MJ, Rajur SB, McLaughlin LW, Hopkins PB. J Am Chem Soc 1995;115:2551.
52. Hopkins PB, Millard JT, Woo J, Weidner MF, Kirchner JJ, Sigurdsson ST, Raucher S. Tetrahedron 1991;47:2475.
53. Sawyer GA, Frederick ED, Millard JT. Chem Res Toxicol 2004;17:1057. [PubMed: 15310237]
54. Kellersberger KA, Yu E, Kruppa GH, Young MM, Fabris D. Analytical Chemistry 2004;76:2438. [PubMed: 15117181]
55. Yu ET, Zhang Q, Fabris D. Journal of molecular biology 2005;345:69. [PubMed: 15567411]
56. Yu E, Fabris D. Analytical Biochemistry 2004;334:356. [PubMed: 15494143]
57. Yu E, Fabris D. Journal of Molecular Biology 2003;330:211. [PubMed: 12823962]
58. Wang Y, Zhang Q, Wang Y. Journal of the American Society for Mass Spectrometry 2004;15:1565. [PubMed: 15519223]
59. Berman HM, Young PR. Annual Review of Biophysics and Bioengineering 1981;10:87.
60. Jesson MI, Johnston JB, Robotham E, Begleiter A. Cancer Research 1989;49:7031. [PubMed: 2531036]

Figure 1.
Structures of the acridine ligands.

Figure 2.
ESI-mass spectrum of 50 uM AS1 with (a) 10 M duplex 1 (b) and 10 M ss2 in 50 mM NH_4OAc .

Figure 3. ESI-mass spectrum of 50 M AS6 with 10 M duplex 1 in 50 mM NH₄OAc. Sodium and potassium adducts are labeled with a triangle, Δ. The enlarged portion of the spectrum shows proposed theoretical structures of the AS6 DNA adducts.

Figure 4.
Relative binding affinities of 50 M acridine ligands with (a) 10 M 14-mer duplexes and (b) 10M 9-mer single strands.

Figure 5. IRMPD spectra of (a) $[ds2 + AS4]^{6-}$ with an irradiation time of 1.0 ms and (b) $[ds2 + AS4]^{5-}$ with an irradiation time of 2.4 ms. The precursor ion is denoted with an asterisk.

Figure 6. IRMPD mass spectra of (a) $[ss2 + AS4]^{3-}$ with an irradiation time of 1.5 ms and (b) $[ss2 + AS4 + ss2]^{4-}$ with an irradiation time of 1.0 ms. The precursor ion is denoted with an asterisk.

Scheme 1.

Proposed mechanism for the alkylation of the guanine base in DNA by AS4.

Duplex 1

Duplex 2

Duplex 3

Duplex 4

Scheme 2.

IRMPD fragments for duplexes 1 to 4 with covalently bound AS4. Slash marks with arrows indicate fragments retaining the AS4 ligand. Bold G's indicate likely sites of alkylation.

Table 1

DNA oligonucleotide sequences used in this study.

Abbreviations	Oligonucleotide Sequences
ds1	5'-GCGCGGAACCGCGC-3' (ssa) 5'-GCGCGGTTCCGCGC-3' (ssb)
ds2	5'-GGCGTCGGCGTCGC-3' (ssa) 5'-GCGACGCCGACGCC-3' (ssb)
ds3	5'-ACGTTACGTTACGC-3' (ssa) 5'-GCGTAACGTAACGT-3' (ssb)
ds4	5'-ATAAACGAAATA-3' (ssa) 5'-TATTTTCGTTTAT-3' (ssb)
ss1	5'-CGCGCGCGC-3'
ss2	5'-ACGTACGTT-3'
ss3	5'-ATATAAATA-3'