High Sensitivity and Fragmentation Specificity in the Analysis of Drug−DNA Adducts by Electrospray Tandem Mass Spectrometry

Paula Iannitti, Margaret M. Sheil,* and Geoffrey Wickham*

Department of Chemistry, University of Wollongong NSW 2522, Australia

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DNA alkylation is a key cellular event in the mechanism of action of clinical anticancer drugs, chemical mutagens, and carcinogens.[1] The nitrogen and oxygen atoms of purines are preferentially alkylated, with the affinity for a particular base depending on the sequence of neighboring bases. It is most likely that the sequence selectivity of the agent is important in determining the nature of the biological response.[2] Methods based on molecular biology have been used extensively to determine the sequence selectivity of alkylation.[3] Such assays are extremely sensitive and may be quantitative; however, they do not yield direct information concerning the structure of the ligand−DNA adducts. NMR spectroscopy[4] and X-ray crystallography[5] enable detailed structural characterization but are time consuming and require large amounts (micromoles) of material. Mass spectrometry should complement these techniques by providing a rapid and sensitive means of determining the sequence selectivity of alkylation and, at the same time, provide some structural detail about the adducts.[6] Several early studies concerned with the sequencing of ligand−DNA adducts by mass spectrometry have involved tandem mass spectrometry (MS/MS) following fast atom bombardment (FAB) ionization.[7] This approach is limited, however, by the relatively poor efficiency of FAB ionization for oligonucleotides.[8] In contrast, electrospray ionization (ESI) has allowed mass measurement of relatively large oligonucleotides, including modified oligonucleotides,[9] an intact plasmid DNA,[10] and noncovalent drug−DNA complexes.[11] Tandem mass spectrometry following electrospray ionization (ESI-MS/MS) has been used for the location of modified bases within oligonucleotides and for the characterization of synthetic oligonucleotides which contain "unnatural" bases in their sequence.[12] To date, however, there have been no reports of the use of ESI-MS/MS for the sequencing of covalent, ligand−oligonucleotide adducts.[13]

Hedamycin (Figure 1) is a naturally-occurring antitumor antibiotic that binds to double-stranded DNA by both intercalation and alkylation. DNA sequencing shows that alkylation occurs exclusively at guanine bases, but with a distinct preference for guanines located in 5′-CGT-3′ and 5′-CGG-3′ sequences.[14] NMR-derived solution structures of hedamycin covalently bound to double-stranded oligonucleotides show that the anthrapyrantrione chromophore of the ligand threads the double helix, placing the two amino sugars in the minor groove and the alkylating bis-epoxide side chain in the major groove.[15] Our previous studies of hedamycin−DNA binding by ESI-MS have confirmed that hedamycin significantly increases duplex stability and provided strong evidence for the formation of specifically, base-paired duplexes in the gas phase.[16] We demonstrate here that ESI-MS/MS can be used to determine unequivocally the sequence selectivity of base alkylation by a DNA-binding antitumor drug, using hedamycin as a model.

ESI-MS/MS spectra of multiply-charged anions of unmodified oligonucleotides are typically characterized by a large number of peaks arising from loss of the nucleobases followed by cleavage of the phosphate ester backbone (in particular, cleavage of the 3′ C−O bond of the depurinated sugar is favored with a preference for loss of adenine compared with guanine).[17] Oligonucleotides containing electron-deficient bases also show enhanced loss of the substituted base.[12a] In MS/MS spectra of $[M\text{-}2H]^{2-}$ ions of 5′-CACGTG-3′, approximately 30 different fragment ions are observed, with no single dominant fragmentation pathway.[17c] In contrast, the ESI-MS/MS spectra of the $[M\text{-}2H]^{2-}$ ion of the single-stranded, hedamycin−5′-CACGTG-

* Authors to whom correspondence should be addressed. FAX: (61-42) 214 287. Phone: M.M.S., (61-42) 213 261; G.W., (61-42) 214 418. E-mail: m.sheil@uow.edu.au and g.wickham@uow.edu.au.

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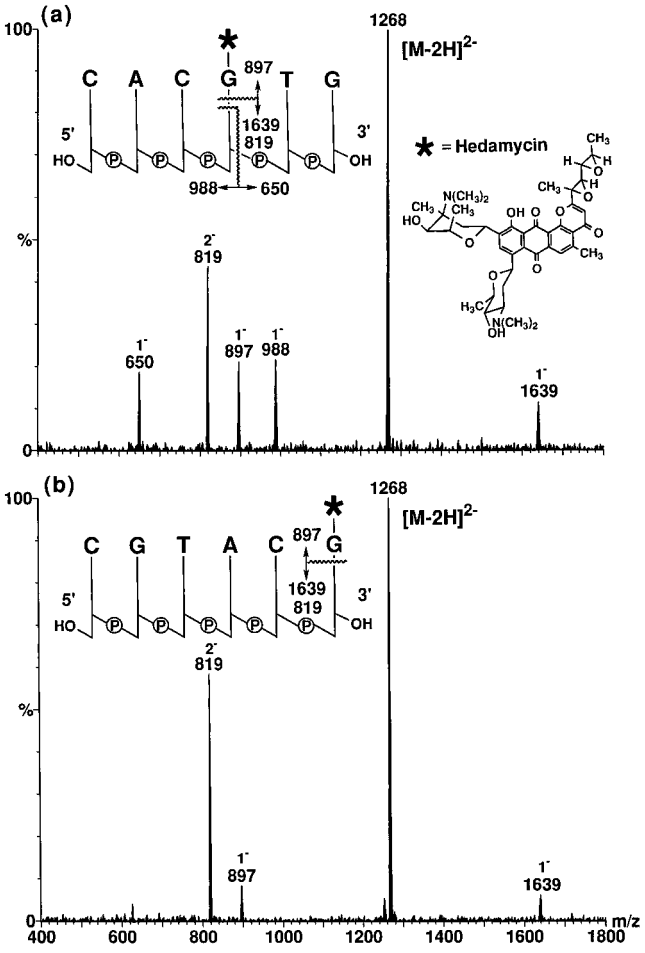

Figure 1. (a) ESI-MS/MS spectrum of the $[M-2H]^{2-}$ ion (*m/z* 1268) of the hedamycin-5′-CACGTG-3′ single-stranded adduct (collision cell voltage, 15 V; argon collision gas, 1.1×10^{-3} mbar). (b) ESI-MS/MS spectrum of $[M-2H]^{2-}$ (*m/z* 1268) ion of hedamycin−5′-CGTACG-3′ single-stranded adduct (collision cell voltage, 15 V; argon collision gas, 1.1×10^{-3} mbar). These data were obtained on a VG Quattro in the negative ion mode and comprised ∼20 scans at a rate of 100 *m/z* per second. The solvent was 50% aqueous methanol and the flow rate was 5 μL/min. Ten microliters of ∼200 pmol/μL solution was injected for each analysis to ensure the best possible signal-to-noise ratio (minimum required would be ∼5−10 times less than this).

3′ adduct under the same collision conditions (Figure 1a) is strikingly simple. Only five product ions are observed, and these all indicate a single major fragmentation pathway which involves the loss of the alkylated guanine via N-glycosidic bond cleavage to give either the doubly-charged ion of the depurinated oligonucleotide at *m/z* 819 or the corresponding singly-charged ion at *m/z* 1639.[18] Cleavage of the 3′ C−O bond of the depurinated nucleotide residue yields two singly-charged oligonucleotide fragments: *m/z* 650 (dinucleotide fragment 3′ to the alkylated guanine) and *m/z* 988 (trinucleotide fragment 5′ to the alkylated guanine). The remaining product ion, *m/z* 897, is the singly-charged hedamycin−guanine adduct. This is consistent with labilization of the glycosidic bond by alkylation and parallels the thermally-induced process that occurs in solution.[19] We have found similar specific fragmentation pathways with a range of other oligonucleotides (*viz.* 5′-CGTACG-3′, 5′-TACGTA-3′, and 5′-CGGTACG-3′). In the case of hedamycin−5′-CGTACG-3′, NMR data[20] had shown that alkylation of the guanine at the 3′ end is preferred, presumably due to the lack of sufficient helical constraints at the end of the duplex. This is reflected in the MS/MS spectrum of the $[M-2H]^{2-}$ ion of the hedamycin-5′-CGTACG-3′ adduct shown in Figure 1b in which only the ions due to loss of hedamycin−guanine (*m/z* 819 and 1639) and hedamycin−guanine adduct (*m/z* 897) are observed. That there are no other fragments is consistent with the location of the ligand at the 3′ terminus. Hence, in all the examples we have examined, the site of binding of the ligand on the oligonucleotide sequence is clearly evident from the MS/MS spectrum.

The spectral simplicity resulting from the combination of high sensitivity of ESI and specific fragmentation in the MS/MS spectra demonstrates the enormous potential of this method for the structural analysis of ligand−DNA adducts. While the analyses reported here were conducted with "off-line" HPLC purification, the technique can, in principle, be expanded to incorporate "on-line" LC-MS/MS, thus providing a rapid means of screening mixtures of alkylated oligonucleotides (which differ in the sequence position of base alkylation) and thereby obtaining some measure of the sequence selectivity of the alkylating agent. We are currently extending this approach to a wider range of alkylating agents and oligonucleotide sequences.

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(18) That both singly- and doubly-charged ions are observed for the same fragmentation pathway indicates that in some cases the hedamycin−guanine adduct is lost as a neutral species. There is a precedent for this in the MS/MS spectra of free oligonucleotides.[17]

(19) This has been demonstrated by ESI and MS/MS spectra of the product from thermally-induced depurination in solution. In solution, H_2O adds to the depurinated DNA prior to strand scission (Iannitti et al., unpublished data).

(20) Pavlopoulos *et al.*, unpublished data.