DNA Damage

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Direct Detection and Mapping of Sites of Base Modification in DNA Fragments by Tandem Mass Spectrometry**

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The mutagenic and carcinogenic potential of a DNA-damaging agent can depend on the chemical nature and sequence selectivity of the modification caused by the agent. For instance, there are mutational hot spots which, if modified and not repaired, are more prone to induce mutations than other positions of the DNA.[1] Currently available methods for detecting and mapping sites of modifications in DNA include polyacrylamide gel electrophoresis (PAGE) and mass spectrometry (MS). PAGE allows mapping and quantitation of some sites of modifications in DNA, while MS provides structural information. However, none of the methods provides information about both the structure and position of the damage.

A newer method developed by Tretyakova et al. utilizes isotopically labeled DNA and MS for mapping and quantifying sites of modifications in DNA,[2] although the method requires prior knowledge of the modifications for designing the labeled DNA, and the information obtained from a single experiment is limited to only one site. Furthermore, all of the available methods require either cleavage or hydrolysis of the DNA, which may be a problem with certain lesions. Herein, we report a simple and"direct" method for the detection and mapping of sites of modifications in DNA. The method does not require any prior DNA cleavage or hydrolysis or the use of radioactive or stable isotopes.

Collision-induced dissociation (CID) of oligonucleotides results in the formation of two major types of ions, the w and a-B ions, the spectra of which are unique and depend on DNA sequence and modifications (Figure 1).[3,4] We have recently used this CID-based approach to sequence normal bases in oligonucleotides.[5] McCloskey and co-workers have used this approach to map a single 5-methylcytosine (5MeC) in an oligonucleotide.[4] However, DNA modifications by chemical agents are typically distributed among multiple sites with varying preferences.[6,7]

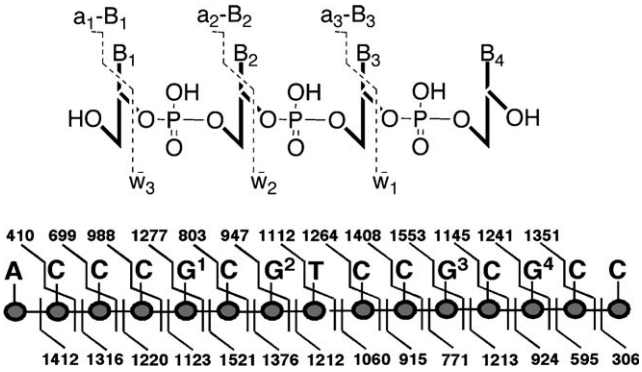
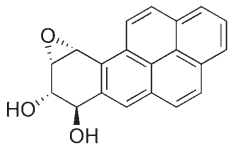
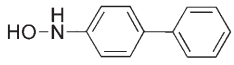


Figure 1. Fragments (a_n-B_n and w_n) resulting from CID of an oligonucleotide. The top and bottom numbers represent a-B and w ions of the unmodified oligonucleotide, respectively.

A 15-base-pair double-stranded oligonucleotide (**1**), which represents a region of exon 5 of the *p53* gene and contains the hot-spot codon 157, was modified with the carcinogen (±)-*anti*-benzo[*a*]pyrene diol epoxide (BPDE) or *N*-hydroxy-4-aminobiphenyl (*N*-OH-4-ABP) under single-hit conditions. The *p53* gene is found to be mutated in more than 50% of cancers.[8] Alkylation of the *p53* gene by BPDE has been linked to the occurrence of lung cancer and *N*-OH-4-ABP to bladder cancer.[9,10] The major alkylation sites of BPDE and *N*-OH-4-ABP are the N2 and C8 atoms of guanine, respectively.[11,12] Alkylation of the noncoding strand of **1** by BPDE and *N*-OH-4-ABP has been previously mapped and therefore provides an excellent system to test our method.[2,6,7]



BPDE



N-OH-4-ABP

5'-ACC CGR^1 G TC CGC GCC
3'-TGG GCG R^{2}AG GCG CGG

1: R^1 = C, R^2 = C
2: R^1 = 5MeC, R^2 = C
3: R^1 = C, R^2 = 5MeC
4: R^1 = 5MeC, R^2 = 5MeC

Accordingly, all experiments and data analysis were limited to the nontranscribed strand of the *p53* gene, in which more than 90% of mutations occur.[6] The quadruply charged species of *m*/*z* 1193.1 eluting at 3.88 min for BPDE (Figure 2a and b) and *m*/*z* 1159.5 eluting at 3.6 min for *N*-

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

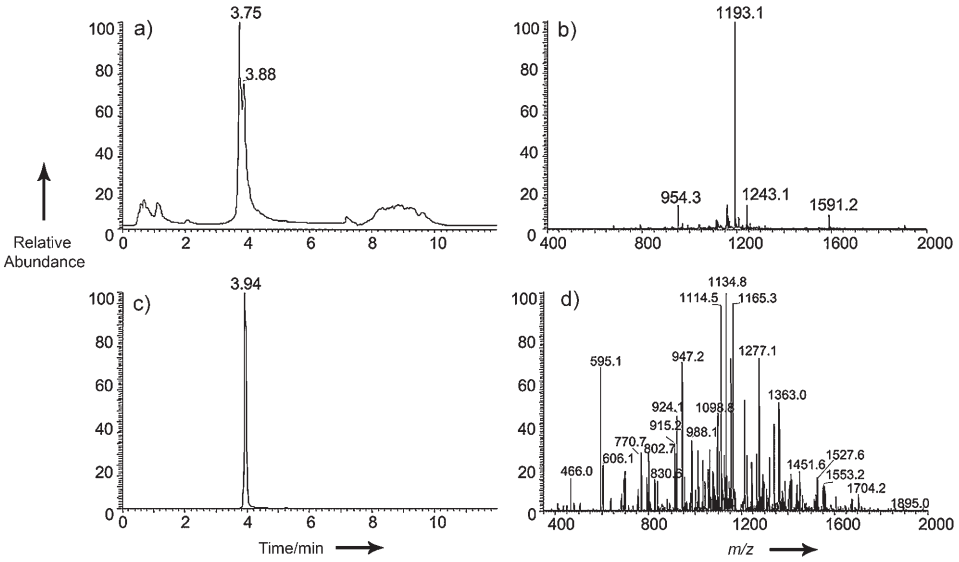

Figure 2. Alkylation of **1** with (±)-*anti*-BPDE. A solution of **1** (40 μM) in Tris-HCl buffer (50 mM) was treated with BPDE (150 μM) for 24 h at 4 °C. a) Total ion current (TIC) chromatogram of **1** treated with (±)-*anti*-BPDE. The peaks at 3.75 and 3.88 min correspond to the unmodified and BPDE-modified noncoding strand of **1**, respectively. b) Mass spectrum of the 3.88 min peak in the chromatogram shown in (a). c) TIC chromatogram of the *m*/*z* 1193.1 ion. d) CID spectrum of the *m*/*z* 1193.1 ion.

OH-4-ABP (Supporting Information) were the major monoalkylated species detected by liquid chromatography (LC)–MS. CID of the *m*/*z* 1193.5 ion generated the spectrum shown in Figure 2d. The calculated *m*/*z* values of the possible w and a-B ions of the unmodified and modified DNA are shown in the Supporting Information.

Alkylation of a base should result in an increase in mass (corresponding to the mass of the alkylating moiety) of all the fragments in the CID spectra that contain the alkylated base, while masses of fragments that do not contain the alkylated base should remain unaltered. Thus, by simply analyzing the masses of the w or a-B ions, the position of a modification in an oligonucleotide can be identified. However, this approach is applicable to only the first and last modification sites (one from each type of ion).

The nontranscribed strand of **1**–**4** contains four guanine residues (G^1–G^4), each of which is a possible alkylation site for both BPDE and *N*-OH-4-ABP. To map and quantify the relative yields of alkylation at multiple sites, an algorithm based on a-B ions (Eqs. (1)–(5); M = modified, UM = unmodified) or w ions (see the Supporting Information) was developed. Using this approach, either the a-B or the w ions can be used to map and quantify the relative yields of modification at multiple sites in an oligonucleotide.

$$a_n\text{-}B_n(M) = \frac{a_n\text{-}B_n(M)}{a_n\text{-}B_n(M) + a_n\text{-}B_n(UM)} \times 100 \quad (1)$$

$$G^{1*}\% = a_6\text{-}B_6(M) \quad (2)$$

$$G^{2*}\% = a_9\text{-}B_9(M) - a_6\text{-}B_6(M) \quad (3)$$

$$G^{3*}\% = a_{12}\text{-}B_{12}(M) - a_9\text{-}B_9(M) \quad (4)$$

$$G^{4*}\% = a_{14}\text{-}B_{14}(M) - a_{12}\text{-}B_{12}(M) \quad (5)$$

We initially tested this approach on an authentic mixture of two oligonucleotides (10 and 20 pmol) with 8-oxo-7,8-dihydroguanine (8-oxoG) at G^1 or G^3. The relative amounts of the adduct at G^1 and G^3 obtained from the w and a-B ions using the method described here gave values similar (1:2) to the prepared mixture (see the Supporting Information). The results also indicated that 0.5–1 pmol of modified oligonucleotide is sufficient for detection, whereas 5–10 pmol of each modified oligonucleotide is necessary for determining the relative yield of alkylation at various sites. The length of oligonucleotides that can be used can be extended by high-resolution MS; however, for larger oligonucleotides overlapping of peaks might become an issue.

The CID spectrum of the monoalkylated noncoding strand of **1** contains a single peak corresponding to the unmodified a_5-B_5 ions (5′-ACCC), which clearly indicates the absence of an alkylation site in this fragment. For the a_6-B_6 ions two peaks were identified, one each for the unmodified and alkylated 5′-ACCCG[1] fragments. Together, these results indicate that there is alkylation at G^1. The percent yield of alkylation at G^1 was calculated from the area under the modified and unmodifed a_6-B_6 ion peaks in the CID spectrum [Eqs. (1) and (2)].

The relative yield of alkylation at the next alkylation site G^2 was determined from the a_9-B_9 or a_{10}-B_{10} ions instead of a_8-B_8 ions (5′-ACCCG[1]CG[2]) because CID of the monoalkylated oligonucleotide did not generate any a_8-B_8 ions. As neither BPDE nor *N*-OH-4-ABP alkylates T or C in this sequence, the relative abundance of the modified and unmodified peaks of the a_8-B_8 ions should be the same as that of the a_9-B_9 ions.[7] Analysis of the CID spectrum revealed the presence of unmodified and modified fragments corresponding to the a_9-B_9 ions. Interestingly, unlike the 5′-ACCCG[1] fragment, the presence of the monoalkylated 5′-ACCCG[1]CG[2]T fragment simply indicates that either G^1 or G^2 is alkylated.

To detect the presence of alkylation at G^2, the percent yields of alkylation were calculated from the areas under the unmodified and modified peaks for a_9-B_9 ions. This value is the sum of alkylation at G^1 and G^2. To obtain the yield of alkyation at G^2, the percent alkylation at G^1 was subtracted from the $G^1 + G^2$ value. Likewise, the relative yields of alkylation at the next alkylation sites, G^3 and G^4, were calculated using a_{12}-B_{12} and a_{14}-B_{14} ions, respectively. Following a similar approach, the relative yields of alkylation at sites

G^1 to G^4 were also calculated using the w ions. The results obtained from the a-B and w ions were remarkably similar, clearly indicating that both the a-B and w ions can be used for mapping sites of modifications in oligonucleotides.

For BPDE, the relative yields of alkylation at G^1 to G^4 are shown in Figure 3 (a-B and w ions). This result is in contrast to an earlier observation that the least-preferred site of alkylation is G^3,[7] probably because of the possibility of the nucleotide excision-repair-mediated DNA cleavage being

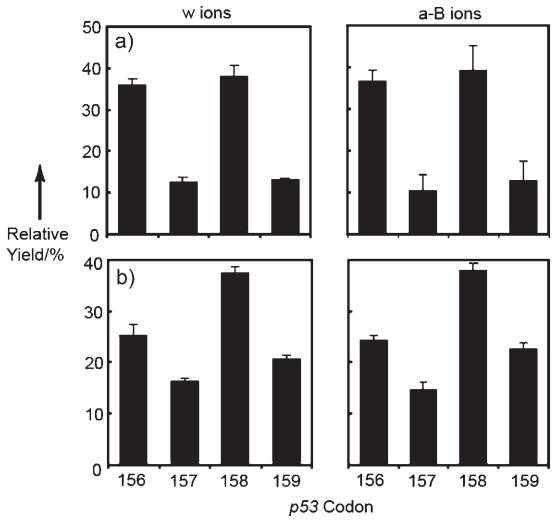

Figure 3. Relative yields of guanine alkylation at various sites in the noncoding strand of **1**. a) A solution of **1** (40 μM) in Tris-HCl buffer (50 mM, pH 7.4) was treated with BPDE (150 μM) for 24 h at 4 °C. b) A solution of **1** (80 μM) in potassium phosphate buffer (10 mM, pH 7.0) containing ethylenediaminetetraacetic acid (1 mM) and acetylsalicylic acid (10 mM) was treated with *N*-OH-4-ABP (400 μM) for 8 h at 37 °C. The relative yields of alkylation were determined from the area under the modified and unmodified a-B or w ion peaks in the CID spectra by using Equations (1)–(10) (see the Supporting Information).

sequence dependent. The relative yields of alkylation at G^1, G^3, and G^4 by *N*-OH-4-ABP are consistent with those reported by Feng et al.[7] The difference in alkylation at G^2 between the two studies may be a result of the higher concentration of *N*-OH-4-ABP used in our experiments.

CpG dinucleotides in exon 5–8 of the *p53* gene are methylated[13] and can affect guanine alkylation. Alkylation by BPDE, *N*-OH-4-ABP, and mitomycin C is significantly increased upon cytosine methylation, whereas for nitrosoureas there is a decrease in alkylation.[6,7,14–16] With the method described here, the presence of 5MeC on the 5′ side of G^2 (**2**) had little or no effect with BPDE, whereas the presence of 5MeC on the opposite strand (**3**) or on both the 5′ side and opposite strand (**4**) increased the relative yield of alkylation by 1.6- and 2.4-fold, respectively (Figure 4), consistent with that reported by Tretyakova et al. using the same sequence.[2] This result suggests that the discrepancy of our data compared to those of Weisenberger and Romano may be a sequence effect;[17] sequence-dependent alkylation of methylated CpG dinucleotides was observed for mitomycin C.[14]

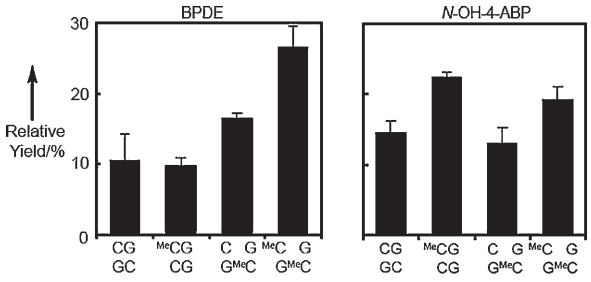

Figure 4. Effect of 5MeC on the relative yield of alkylation of guanine at codon 157 (G^2) of the *p53* gene by BPDE and *N*-OH-4-ABP. Oligonucleotides **1**–**4** were alkylated with BPDE or *N*-OH-4-ABP by following the same procedure as that described in the legend of Figure 3. The relative yields of alkylation were determined from the areas under the modified and unmodified a-B ion peaks using Equations (1)–(5).

For *N*-OH-4-ABP, the effect of 5MeC was less pronounced; 5MeC on the 5′ side (**2**) or on both the 5′ side and on the opposite strand (**4**) of G^2 increased the relative yield of alkylation at G^2 by only 1.6- and 1.3-fold, respectively (Figure 4). The contrasting effects of the position of 5MeC on DNA alkylation by BPDE and *N*-OH-4-ABP may be because of their modes of alkylation, noncovalent association with DNA, or local changes in the DNA structure.

Another aspect of the method described here is its ability to provide regioselectivity of the alkylation. CID of DNA also results in the elimination of the base as a negatively charged species that can be detected in the CID spectrum.[3,4] Accordingly, analysis of the CID spectrum of BPDE-adducted DNA revealed the presence of three peaks (*m*/*z* 452, 434, and 416) corresponding to the BPDE-G adduct and its dehydration products (see the Supporting Information). LC–MS^3 of the *m*/*z* 452 peak provided the CID spectrum shown in Figure 5, in which the *m*/*z* 300 peak resulting from the fragmentation of the C2 and N2 bond of the BPDE-G adduct is indicative of an N2 adduct and not an O6 adduct, because an O6 adduct of guanine should give a peak at *m*/*z* 301. This result is consistent with a previous report that BPDE alkylates the N2 atom of guanine.[18]

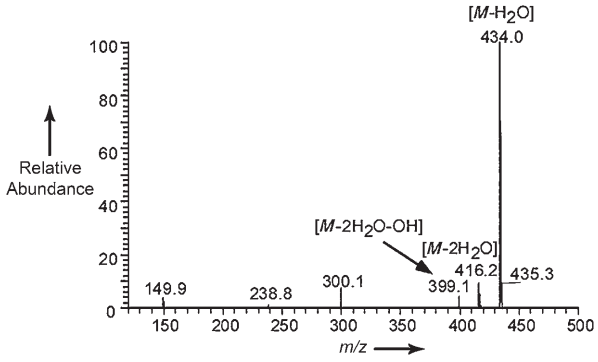

Figure 5. LC–MS^3 spectra of the guanine–BPDE adduct (*m*/*z* 452). BPDE-alkylated **1** was subjected to LC–MS/MS analysis as shown in Figure 2. The *m*/*z* 452 ion in the CID spectrum (Figure 2d) was further subjected to CID (*m*/*z* 1193.5 and 452 at an energy setting of 35).

In conclusion, we have presented a direct method for the detection and mapping of DNA alkylation. BPDE and *N*-OH-ABP modifications were analyzed and the effects of 5MeC on adduct formation were elucidated. This method may be applicable to a wide variety of DNA modifications including alkylation and oxidatively generated, nonlabile, bulky adducts. No cleavage or hydrolysis of the DNA is necessary, samples can be directly loaded onto a LC–MS system, and the use of radioactive or stable isotopes is not required. The method can be extended to the analysis of multiple DNA fragments simultaneously in a single experiment. Multiple DNA adducts in the same DNA molecule or in various DNA molecules can also be analyzed, thus providing opportunities to study chemistry in the relevant oligonucleotides instead of nucleosides and model compounds.[19]

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[1] E. C. Friedberg, G. C. Walker, W. Siede, R. D. Wood, R. A. Schultz, T. Ellenberger, *DNA Repair and Mutagenesis*, 2nd ed., American Society for Microbiology, Washington, DC, **2005**.
[2] N. Tretyakova, B. Matter, R. Jones, A. Shallop, *Biochemistry* **2002**, *41*, 9535 – 9544.
[3] S. A. McLuckey, S. Habibi-Goudarzi, *J. Am. Chem. Soc.* **1993**, *115*, 12085 – 12095.
[4] J. Ni, C. Pomerantz, J. Rozenski, Y. Zhang, J. A. McCloskey, *Anal. Chem.* **1996**, *68*, 1989 – 1999.
[5] J. Y. Choi, G. Chowdhury, H. Zang, K. C. Angel, C. C. Vu, L. A. Peterson, F. P. Guengerich, *J. Biol. Chem.* **2006**, *281*, 38244 – 38256.
[6] M. F. Denissenko, A. Pao, M. Tang, G. P. Pfeifer, *Science* **1996**, *274*, 430 – 432.
[7] Z. Feng, W. Hu, W. N. Rom, F. A. Beland, M. S. Tang, *Biochemistry* **2002**, *41*, 6414 – 6421.
[8] A. A. Levesque, A. Eastman, *Carcinogenesis* **2007**, *28*, 13 – 20.
[9] G. P. Pfeifer, M. F. Denissenko, M. Olivier, N. Tretyakova, S. S. Hecht, P. Hainaut, *Oncogene* **2002**, *21*, 7435 – 7451.
[10] G. Talaska, A. Z. al-Juburi, F. F. Kadlubar, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 5350 – 5354.
[11] F. A. Beland, D. T. Beranek, K. L. Dooley, R. H. Heflich, F. F. Kadlubar, *Environ. Health Perspect.* **1983**, *49*, 125 – 134.
[12] W. G. Humphreys, F. F. Kadlubar, F. P. Guengerich, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 8278 – 8282.
[13] S. Tornaletti, G. P. Pfeifer, *Oncogene* **1995**, *10*, 1493 – 1499.
[14] V. S. Li, M. S. Tang, H. Kohn, *Bioorg. Med. Chem.* **2001**, *9*, 863 – 873.
[15] B. H. Mathison, B. Said, R. C. Shank, *Carcinogenesis* **1993**, *14*, 323 – 327.
[16] M. Rajesh, G. Wang, R. Jones, N. Tretyakova, *Biochemistry* **2005**, *44*, 2197 – 2207.
[17] D. J. Weisenberger, L. J. Romano, *J. Biol. Chem.* **1999**, *274*, 23948 – 23955.
[18] W. Xue, D. Warshawsky, *Toxicol. Appl. Pharmacol.* **2005**, *206*, 73 – 93.
[19] Note added in proof: After submission of this manuscript, Vouros et al. published a method of analysis of modified oligonucleotides using CID that has some similarity to our method, but which is not applied to chromatographically unseparated mixtures. W. Xiong, J. Glick, Y. Lin, P. Vouros, *Anal. Chem.* **2007**, *79*, 5312 – 5321.

