DNA adduct profiles: chemical approaches to addressing the biological impact of DNA damage from small molecules

Shana J Sturla

Diverse small molecules alkylate DNA and form covalently linked adducts that can influence crucial biological processes, contributing to toxicity and mutation. Understanding the chemical reactivity dictating DNA alkylation and interactions of adducts with biological pathways can impact disease prevention and treatment. The ambident reactivity of DNA-alkylating small molecules, and of DNA itself, often results in formation of multiple adducts. Determining which structures impart biological responses is important for understanding the underlying relationships between small-molecule structure and biology. With application of sensitive and structure-specific experimental and analytical methodology, such as heteronuclear NMR spectroscopy and mass spectrometry, there are increasing numbers of studies that evaluate DNA alkylation from the perspective of resulting adduct profiles. DNA adduct profiles have been examined for both exogenous and endogenous reactive small molecules. Examples of recent findings are in the areas of tobacco-specific carcinogens, lipid peroxidation products, environmental and dietary chlorophenols, and natural-product-derived antitumor therapies. As more profile data are obtained, correlations with biological impact are being observed that would not be identified by a simplified single agent/single adduct approach.

Addresses
Department of Medicinal Chemistry and The Cancer Center, University of Minnesota, 308 Harvard Street SE, Minneapolis, MN 55455, USA

Corresponding author: Sturla, Shana J (sturl002@umn.edu)

Current Opinion in Chemical Biology 2007, **11**:293–299

This review comes from a themed issue on
Combinatorial chemistry and molecular diversity
Edited by Gregory A Weiss and Richard Roberts

1367-5931/$ – see front matter

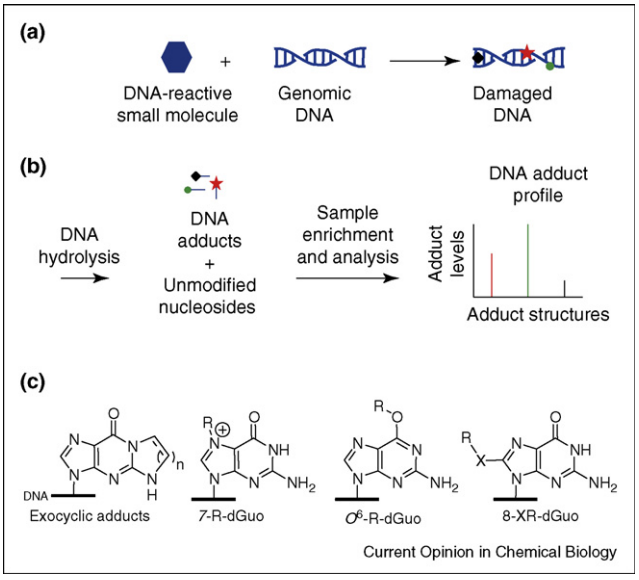
DOI 10.1016/j.cbpa.2007.05.021

Introduction

Cellular reactions involving DNA and electrophilic small molecules can result in structural damage to DNA in the form of covalent DNA adducts. DNA adducts are potential substrates for repair enzymes or polymerases, or they can block these activities, triggering biological responses including cell death and mutation. Diverse chemical sources, structures and biological consequences are associated with DNA adduct formation. Furthermore, when considering DNA adducts on the nucleoside level, as is the focus of this review, a single small molecule or class of molecules can contribute to the formation of multiple adducts (Figure 1). Traditionally, the biological impact of DNA damage has often been probed on the basis of a single electrophile giving rise to a single nucleoside adduct, frequently the most abundant for a given alkylating agent. However, minor nucleoside adducts can have more significant biological impact than a more abundant adduct. For example, the more abundant adduct could be a substrate for an efficient repair enzyme, whereas the minor adduct might persist and be highly mutagenic. By contrast, if the persistence of different adducts is similar, an abundant adduct could be an excellent surrogate biomarker for a difficult to detect minor lesion. Understanding how a spectrum or profile of adducts responds to changing biological situations therefore has the potential to provide important mechanistic information that could be obscured when focusing on a single adduct. With the ultimate goal of evaluating changing adduct patterns or profiles to assess disease risk or therapeutic effectiveness, experimental approaches require a detailed understanding of the ambident reactivity of alkylating agents and nucleobases, as well as methods to evaluate multiple adducts simultaneously.

DNA-alkylating agents can be intrinsically reactive, but it is more typical that enzyme-mediated bioactivation is required to produce biologically reactive intermediates. Examples of relevant chemical functionalities include aldehydes, aryl amines, cyclopropanes, enones and enals, epoxides, furans, nitrosamines, phenols, polycyclic aromatic hydrocarbons and quinones. The four nucleotides exhibit ambident reactivity such that multiple positions of each nucleotide can be alkylated; representative classes of adduct structures are illustrated in Figure 1c. Further differences in reactivity can result from local DNA sequence and chromosome structure. It would be impossible to provide a comprehensive review of this broad area in a brief article; therefore, this review will highlight a small selection of studies with a focus on recent results and current experimental approaches in the identification and measurement of adducts and their profiles to understand the chemistry and biology of DNA-alkylating small molecules. Three classes of DNA-alkylating small molecules — tobacco nitrosamines, chlorophenols and lipid peroxidation products — will illustrate ongoing studies. The discussion will close with an outlook on future prospects.

Figure 1

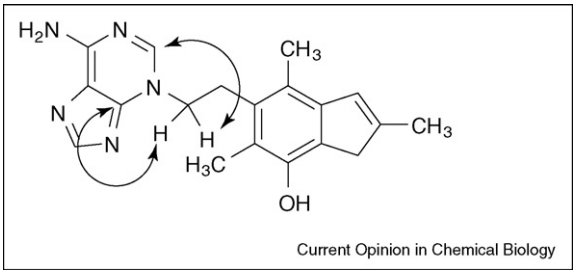


(a) Electrophilic small molecules damage cellular DNA by forming small amounts of covalent DNA adducts, with most genomic DNA remaining unmodified. Biological responses include DNA repair, toxicity and mutation. **(b)** Evaluating profiles of nucleoside adducts is an experimental approach to elucidate mechanisms and implement disease biomarkers. DNA isolation and hydrolysis yields a mixture of modified and unmodified nucleosides. Enrichment and quantitative analysis reveals the DNA adduct profile for specific small molecules under a given biological condition. **(c)** Representative adduct structures are illustrated for the example of guanine. R = alkyl; n = 1, 2; X = O, NH.

Chemical analysis of DNA adducts

A challenge in DNA adduct structure identification is that adducts generally are formed in complex mixtures and at exceedingly low levels. They can be chemically unstable and lack clearly diagnostic spectroscopic signals, particularly for ^{1}H NMR. Heteronuclear correlation NMR spectroscopy data and chemical shifts from 1D ^{13}C NMR experiments are useful for determining the position of connectivity to a nucleoside. For such experiments, sufficient quantities of adduct can be purified from optimized nucleoside reactions or independent synthetic approaches. Harris and co-workers recently reported, for ^{15}N-labeled adducts of the carcinogenic mycotoxin aflatoxin, NMR data that contributed to solving a long-standing question regarding the structures of formamidopyrimidine adducts [1•]. Adducts were determined to be geometrical isomers of atropisomers about a single nitrogen atom, rather than regio- or structural isomers, as previously proposed. In a recent example from our laboratory, ^{13}C chemical shift and ^{13}C–^{13}C coupling constants for a ^{13}C-labeled chloroquinone adduct were used to identify its chemical structure [2]. Positions of base alkylation can often be distinguished on the basis of long-range ^{1}H–^{13}C heteronuclear bond correlations, as illustrated by the example of a DNA adduct of the drug acylfulvene (Figure 2) [3]. To determine the influences of

Figure 2



3-substituted adenine adduct of the drug acylfulvene. Arrows illustrate ^{1}H–^{13}C correlations obtained by HMBC NMR analysis, supporting the assignment of the adduct chemical structure.

adduct formation on perturbing the DNA duplex, synthetic oligonucleotides that site-specifically incorporate adducts have been characterized structurally by X-ray crystallography or NMR spectroscopy [4]. Furthermore, site-specifically modified oligonucleotides are valuable chemical tools for understanding the potential biological impact of DNA adducts when used as substrates in polymerase-mediated DNA synthesis reactions [5•], and for gaining structural and mechanistic information regarding translesion DNA synthesis [6,7].

DNA adduct levels as low as one damaged base in 10^{6}–10^{12} undamaged bases can be significant and reproducible, with definite biological consequences. Sample size limitations may produce as little as 1 μg of DNA, requiring analysis capabilities on the order of femtomoles or, ideally, less. Extremely sensitive structure-specific methods are therefore required. Post-labeling, fluorescence, electrochemical and immunological detection-based methods can have excellent sensitivity for the analysis of certain DNA adducts, but might not be well suited to multiple adducts, to evaluate adducts with significant differences in chemical properties or to obtain information regarding chemical identity. The technique of ^{32}P-post-labeling involves enzyme-mediated labeling of the DNA hydrolysate with ^{32}P-ATP and subsequent separation of modified nucleotides (traditionally by 2D thin-layer chromatography), producing radioactive spots indicative of DNA adducts. Although broadly applicable, it is not readily automated, relies on the use of ^{32}P radioisotope labeling and may present difficulties in distinguishing specific adducts in a complex mixture of adducts. For these reasons, mass spectrometry (MS) is emerging as the method of choice for DNA adduct analysis in many cases [8••]. It is readily automated for high-throughput analysis and is rich in information regarding chemical structure. Accurate quantitation of absolute and relative adduct levels can be accomplished using stable isotope dilution methods. In this approach, DNA is isolated from sources such as cultured cells or animal tissue, and a stable-isotope-labeled standard or

mixture of standards, which have identical chromatographic properties to the analyte but differ by at least two or three mass units, is combined with the DNA under investigation. DNA hydrosylates are composed of a small amount of adduct and high levels of unmodified nucleosides that can suppress the analyte signal (Figure 1). Therefore, sample enrichment techniques, such as solid-phase extraction or molecular weight cutoff filters, are typically used. By LC-MS/MS, ratios of labeled to unlabeled adducts are determined and levels of chemically distinct adducts can be compared (Figure 1).

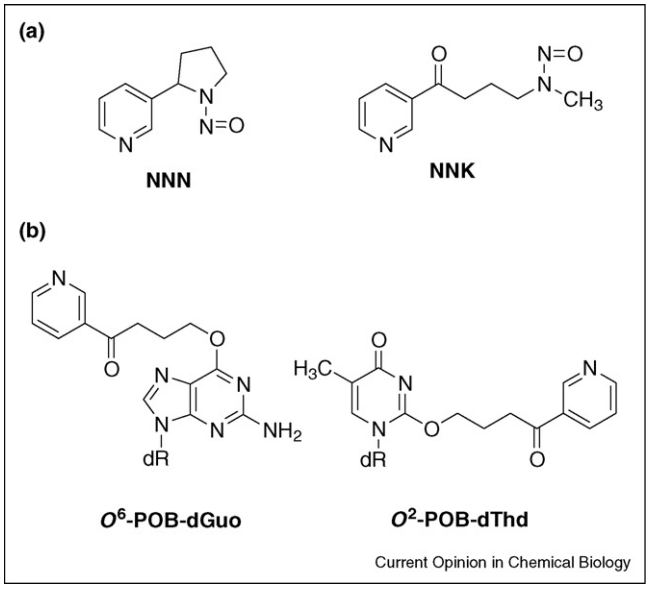
Exogenous and endogenous carcinogen–DNA adducts

In the past several years, hundreds of studies with a wide range of objectives have addressed issues related to complex DNA adduct mixtures. Results include identification of adduct structure, elucidation of adduct-specific contributions to carcinogenesis and toxicity mechanisms, and implementation of DNA adducts as *in vivo* chemical biomarkers. This section will briefly highlight three examples of carcinogens of exogenous and endogenous origin: tobacco-specific nitrosamines, lipid peroxidation products and chlorophenols.

Tobacco-derived nitrosamines

The tobacco-specific nitrosamines N′-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are human carcinogens found in tobacco and its smoke (Figure 3). Oxidative bioactivation of NNN and NNK, and subsequent reaction with DNA, produces pyridyloxobutyl DNA (POB-DNA) (Figure 3) by reacting at multiple sites in DNA, including the 7- and O^6-positions of dGuo, and the O^2-position of dCyt and Thd. Of these, O^6-POB-dGuo is mechanistically implicated in NNK carcinogenesis, but less is known regarding more recently identified lesions [9•,10,11]. In a cell-free system, the relative reactivity of each nucleobase toward activated NNK was determined to be 1:23:6:4 for O^6-POB-dGuo:7-POB-dGua:O^2-POB-Cyt:O^2-POB-Thd, with only small variations when relative amounts of DNA and the electrophile were changed. However, *in vivo* data (i.e. analysis of DNA samples from carcinogen-exposed animals) indicate that the adduct burden in specific tissues does not correlate with the intrinsic reactivity of the alkylating intermediate and suggest that the O^6-adduct can be preferentially eliminated by repair processes in the rat liver versus lung [9•,10]. Furthermore, adduct profiles from animals chronically treated with enantiomers of NNAL — a metabolite of NNK — indicate stereospecific NNAL retention in tissue and re-conversion to NNK [9•,10]. These relationships were indicated by tracking profiles of adducts rather than a single test.

Figure 3

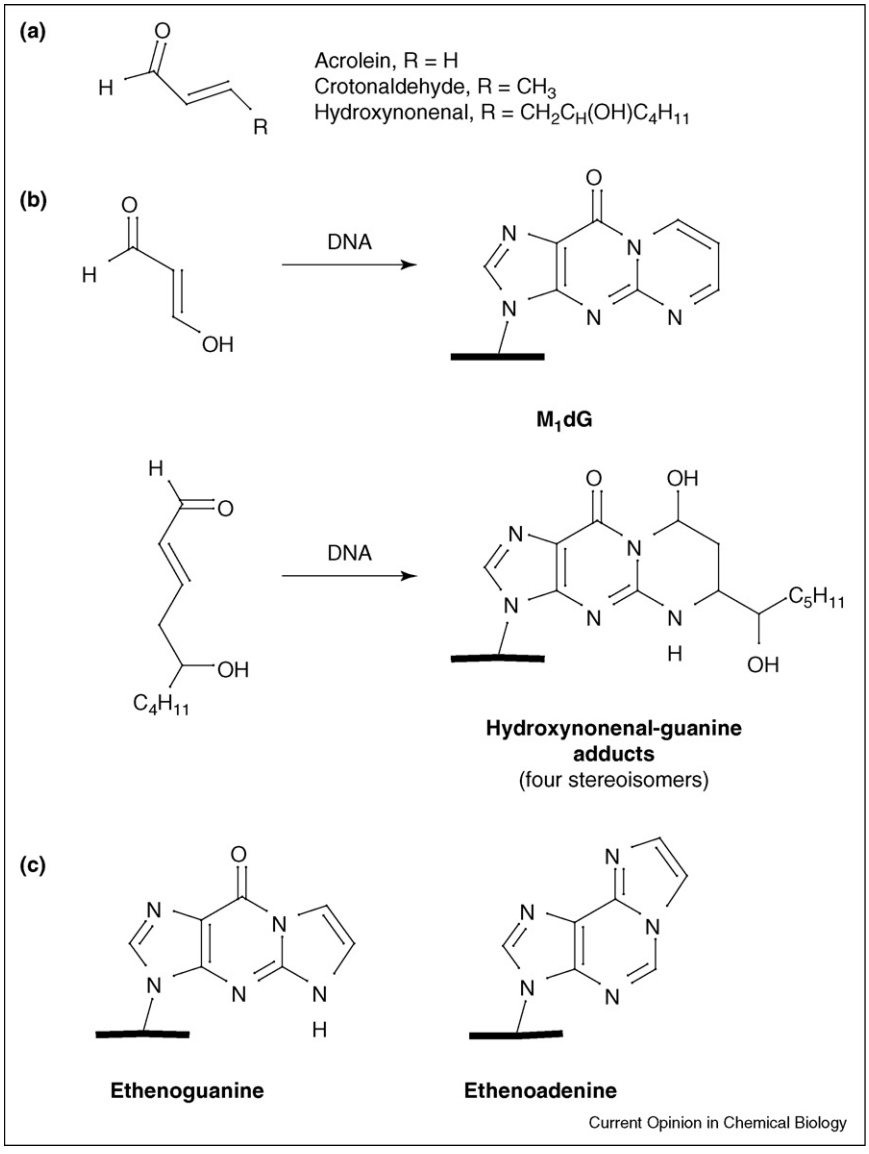


(a) The tobacco-specific nitrosamines NNN and NNK are metabolically activated to produce a pyridyloxobutylating (POB) species that modifies DNA at multiple positions. **(b)** Two representative POB–DNA adducts are illustrated. Multiple POB–DNA adducts have been profiled in cell-free systems and in specific tissue of carcinogen-treated animals. Data indicate a biological role for tissue-specific retention and repair of adducts.

Lipid peroxidation products

Endogenous reactive chemicals are a major source of DNA damage and can contribute to diseases and conditions such as Alzheimer's disease, asthma, rheumatoid arthritis, diabetes, multiple sclerosis, Parkinson's disease and stroke [12]. The concepts and approaches established to study xenobiotic-induced DNA damage apply to correlating relationships of diseases to inflammation, oxidative damage and lipid peroxidation [13]. Lipid peroxidation, resulting from reactions of reduced oxygen species and polyunsaturated fatty acids, is a source of secondary DNA-reactive electrophilic small molecules (e.g. acrolein, crotonaldehyde, 4-hydroxynonenal [4-HNE] and malondialdehyde; Figure 4a). In typical lipid-peroxidation-derived damage, minor-grove modification results in adducts with an added nucleobase ring (i.e. exocyclic adducts; Figures 1c and 4b,c) that are frequently mutagenic and unstable. In a mouse model engineered to produce elevated reactive nitrates (NO, ONOOCO2−, N_2O_3, NO_2), a 12-adduct profile includes exocyclic adducts as well as markers for direct DNA–reactive oxygen species (ROS) interactions and nucleobase deamination [14••]. In this study, increases in lipid-peroxidation-associated etheno adducts correlated with increased inflammation; however, had a single direct oxidation product been monitored, a positive association would not have been revealed [14••]. In a mouse model with cyclooxygenase-2 overexpression, multiple heptanone-etheno adducts were profiled for the first time in mammalian tissue DNA [15••]. Elevations in adduct

Figure 4



(a) Representative α,β-unsaturated carbonyl compounds derived from lipid peroxidation. **(b)** Reactions producing exocyclic DNA adducts. **(c)** Exocyclic etheno adducts.

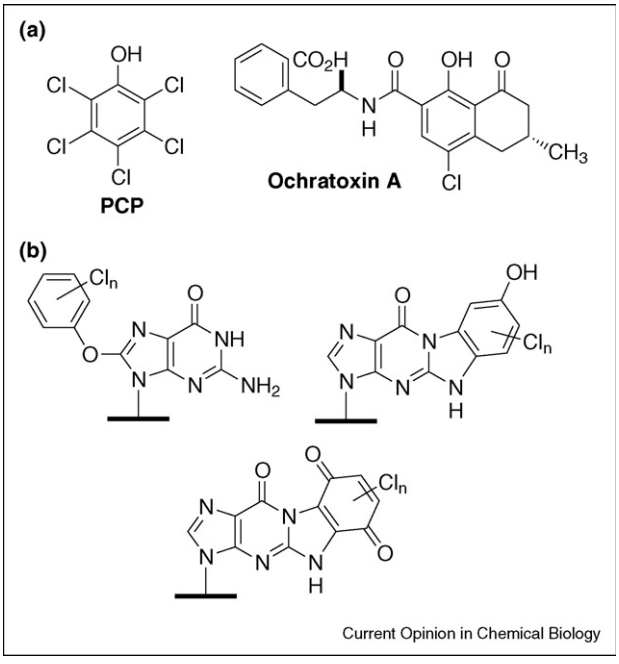
levels were associated with increased levels of cyclooxygenase-2. Finally, although no significant differences were observed between the small groups studied, as little as 24 amol of hydroxynonenal guanine adduct alone was compared in postmortem brains from normal and Alzheimer's disease subjects [16]. Adducts resulting from a mixture of lipid peroxidation products can be complicated if the secondary products react with one another before alkylating DNA. Evidence for the possibility of such 'conjugate' adducts has been obtained in cell-free models using calf thymus DNA [17,18]. Further elucidation of molecular mechanisms for how etheno adducts contribute to mutation will require an understanding of how diverse cellular polymerases and repair enzymes process these lesions. Certain polymerases can be more efficient at synthesis past a specific lesion than the corresponding unmodified base [19]. A comparison of steady-state kinetics and sequence analysis of DNA synthesis products for multiple polymerases reveals that human pol η efficiently synthesizes past the ethenoguanine adduct (Figure 4c) in an oligonucleotide [20]. For ethenoadenine, a combination of Pol ι and Pol ξ efficiently synthesize past this adduct in an error-free manner [19]. It has been known that base glycosylases could be involved in exocyclic adduct repair, but ethenoadenine was recently found to be directly repaired by *Escherichia coli* AlkB and

human ABH3 *in vitro* [21]. These repair enzymes could therefore play a role in mitigating the mutagenic potential of lipid peroxidation adducts.

Environmental and dietary chlorophenols

Chlorophenols are widespread environmental and food contaminants resulting from their application as pesticides, herbicides and wood preservatives; in addition, they can be breakdown products of related agents or are natural products. Examples include pentachlorophenol (PCP), 2,4,6-trichlorophenol, 2,4-dichlorophenol, other isomers and the mycotoxin ochratoxin A (Figure 5). As a class, chlorophenols are acute toxins and possible human carcinogens. Their metabolism results in increases in ROS, which probably play a role in chlorophenol toxicity and carcinogenesis [22]. Chlorophenol carcinogenesis could also be linked with metabolic activation to phenoxyl radicals and quinones that covalently bind to DNA [23–26]. These direct pathways are of particular interest because they offer candidate exposure biomarker profiles that are agent specific and potentially useful for prevention measures. For ochratoxin A [27], comparisons of adduct levels in reactions with ochratoxin alone versus with microsomes indicate that a quinone metabolite generates adducts that could be important for carcinogenicity. Likewise, quinone metabolites of simple chlorophenols react with DNA and multiple adducts can be formed. Illustrated in Figure 5b are representative chloroquinone–DNA adducts [2,28••]. *In vitro* reactivity profiles for chlorophenol metabolites indicate the potential for direct DNA adduction by oxygen-based radicals, carbon-based radicals and quinones. In future studies, profiles from cells and animal tissues, and mutational assays are expected to reflect the biological relevance of direct chlorophenol–DNA adducts.

Figure 5



(a) Examples of chlorophenols of environmental and dietary origin. **(b)** Structures of representative direct chlorophenol–DNA damage products resulting from phenoxy radical and quinone metabolites, illustrated for dGuo as the reacting base.

Conclusions

DNA adduct profiles reflect selectivities in nucleoside reactions, but no information is gained regarding the influence of DNA sequence context on alkylation profiles. This could be an important parameter in determining the tumor-cell selectivity of DNA-alkylating agents and the carcinogenic potential of adduct-derived mutations [29]. There are many examples of sequence selection in DNA alkylation, notably by natural-product-derived DNA-alkylating antitumor agents [29,30]. Achieving selective toxicity to malignant cells and resistance by healthy cells is difficult, and DNA-alkylation selectivity might therefore be a factor that dictates therapeutic index. Sequence selectivity has been addressed in model systems using defined oligonucleotide sequences and modification sites determined by MS [31], or using gel sequencing approaches in cases in which the modification is base labile, leading to strand breaks at the position of modification. Taking the question of sequence selectivity a step further, researchers recently addressed the potential influence of chromatin structure, demonstrating that, regardless of whether alkylation is carried out with free DNA or nucleosome core particles, sequence selectivity and alkylation efficiencies were similar [32•]. It will be important to continue exploring the contributions of DNA packaging to reactivity and determine whether these initial observations hold for DNA alkylation chemistry that gives rise to mutagenic rather than cytotoxic lesions and whether chromatin structure imparts selectivity to reactions with non-sequence-selective agents. For example, the natural-product-derived acylfulvenes are potential antitumor agents that that modify the 3-position of dAde to generate thermally unstable adducts, but resulting patterns of DNA strand scission indicate that the reaction occurs independent of sequence [3,33].

There is a clear need for experimental approaches that are faster, can handle increased numbers of samples and can use smaller sample sizes. Applications of alternative chromatographic methods such as capillary electrophoresis (CE) are emerging as potential improvements. Although significant technical challenges for CE-MS adduct analysis (i.e. orders of magnitude differences in CE versus MS sample volumes and approaches for quantitation) need to be addressed, recent advanced applications of CE-MS, such as those focused on protein analysis [34], demonstrate its potential for quantitation [35] of modified nucleic acids ([36]; see also Update).

Accelerator MS, with the capacity to detect attomole (10^{-18}) levels of many isotopes (i.e. ^{3}H, ^{14}C), is among the most sensitive DNA adduct detectors [37]. A significant drawback has been the need to dose with labeled small molecules, but recent examples illustrate that radio-post-labeling is a potential solution [38]. Finally, a fundamental limitation in adduct detection is that amplification methods (i.e. PCR) cannot be used to enrich samples for DNA analysis because adduct-specific information would be lost. Nucleoside-based probes that pair specifically with biologically formed adducts, rather than canonical nucleosides, might have potential for the development of amplification-based strategies to study DNA damage in the future [39].

Increasingly large profiles are expected to result from biological samples exposed to a realistic exogenous and endogenous small-molecule cocktail. An emerging strategy to profile the resulting adductome involves neutral loss analysis, a MS technique that automatically monitors a common fragmentation pattern (e.g. loss of deoxyribonucleoside) associated with a pattern of adducts [40••]. Future nucleoside-based biomarkers of chemical exposure and drug therapy will clearly expand the current limited profiles of adducts to wide arrays of mixed adducts from multiple sources and of unknown identity. Whereas these data are expected to have great practical application, long-term strategies aiming to understand biological responses on the basis of detailed chemical and biochemical mechanisms of small molecule–DNA reactivity will continue to be crucial for ultimately understanding and predicting biological impact on the basis of small-molecule structure.

Update

Zhu and co-workers [41] recently reported the first example of pre-concentrating oligonucleotides on-line for the analysis of adducts (derived from a metabolite of the carcinogen benzo[a]pyrene) by capillary zone electrophoresis-MS.

Acknowledgements

The author would like to thank Lisa Peterson for helpful suggestions and the National Institutes of Health (CA 108604) for support.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Brown KL, Deng JZ, Iyer RS, Iyer LG, Voehler MW, Stone MP, Harris CM, Harris TM: **Unraveling the aflatoxin-FAPY conundrum: structural basis for differential replicative processing of isomeric forms of the formamidopyrimidine-type DNA adduct of aflatoxin B1**. *J Am Chem Soc* 2006, **128**:15188-15199.
An example of the use of correlation NMR spectroscopy for the identification of DNA adduct structures.

2. Nguyen TN, Bertagnolli AD, Villalta PW, Buhlmann P, Sturla SJ: **Characterization of a deoxyguanosine adduct of tetrachlorobenzoquinone: dichlorobenzoquinone-1,N2-etheno-2′-deoxyguanosine**. *Chem Res Toxicol* 2005, **18**:1770-1776.

3. Gong J, Vaidyanathan VG, Yu X, Kensler TW, Peterson LA, Sturla SJ: **Depurinating acylfulvene-DNA adducts: characterizing cellular chemical reactions of a selective antitumor agent**. *J Am Chem Soc* 2007, **129**:2101-2111.

4. Xu W, Merritt WK, Nechev LV, Harris TM, Harris CM, Lloyd RS, Stone MP: **Structure of the 1,4-Bis(2′-deoxyadenosin-N(6)-yl)-2S,3S-butanediol intrastrand DNA cross-link arising from butadiene diepoxide in the human N-ras codon 61 sequence**. *Chem Res Toxicol* 2007, **20**:187-198.

5. • Guengerich FP: **Interactions of carcinogen-bound DNA with individual DNA polymerases**. *Chem Rev* 2006, **106**:420-452.
A review focusing on strategies involving site-specifically modified oligonucleotides and individual DNA polymerases that can be used to evaluate mechanisms of mutagenesis.

6. Eoff RL, Irimia A, Egli M, Guengerich FP: ***Sulfolobus solfataricus* DNA polymerase Dpo4 is partially inhibited by "wobble" pairing between O6-methylguanine and cytosine, but accurate bypass is preferred**. *J Biol Chem* 2007, **282**:1456-1467.

7. Batra VK, Shock DD, Prasad R, Beard WA, Hou EW, Pedersen LC, Sayer JM, Yagi H, Kumar S, Jerina DM, Wilson SH: **Structure of DNA polymerase beta with a benzo[c]phenanthrene diol epoxide-adducted template exhibits mutagenic features**. *Proc Natl Acad Sci USA* 2006, **103**:17231-17236.

8. •• Singh R, Farmer PB: **Liquid chromatography-electrospray ionization-mass spectrometry: the future of DNA adduct detection**. *Carcinogenesis* 2006, **27**:178-196.
A clear and thorough review that comprehensively references and discusses many of the earlier studies contributing to the experimental strategies and conclusions expressed in this review.

9. • Lao Y, Yu N, Kassie F, Villalta PW, Hecht SS: **Formation and accumulation of pyridyloxobutyl DNA adducts in F344 rats chronically treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and enantiomers of its metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol**. *Chem Res Toxicol* 2007, **20**:235-245.
This study demonstrates detailed mechanistic information related to the stereospecific accumulation of NNAL obtained by profiling carcinogen-specific DNA adducts *in vivo*.

10. Lao Y, Villalta PW, Sturla SJ, Wang M, Hecht SS: **Quantitation of pyridyloxobutyl DNA adducts of tobacco-specific nitrosamines in rat tissue DNA by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry**. *Chem Res Toxicol* 2006, **19**:674-682.

11. Sturla SJ, Scott J, Lao Y, Hecht SS, Villalta PW: **Mass spectrometric analysis of relative levels of pyridyloxobutylation adducts formed in the reaction of DNA with a chemically activated form of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone**. *Chem Res Toxicol* 2005, **18**:1048-1055.

12. West JD, Marnett LJ: **Endogenous reactive intermediates as modulators of cell signaling and cell death**. *Chem Res Toxicol* 2006, **19**:173-194.

13. Liebler DC: **The poisons within: application of toxicity mechanisms to fundamental disease processes**. *Chem Res Toxicol* 2006, **19**:610-613.

14. •• Pang B, Zhou X, Yu H, Dong M, Taghizadeh K, Wishnok JS, Tannenbaum SR, Dedon PC: **Lipid peroxidation dominates the chemistry of DNA adduct formation in a mouse model of inflammation.** *Carcinogenesis* 2007, in press.
In this study, adduct profiles are used to compare changes in adducts representing diverse DNA damage pathways and correlate with them elevated ROS levels associated with inflammation. Positive correlations were observed in certain cases, notably lipid-peroxidation-derived adducts.

15. •• Williams MV, Lee SH, Pollack M, Blair IA: **Endogenous lipid hydroperoxide-mediated DNA-adduct formation in min mice**. *J Biol Chem* 2006, **281**:10127-10133.
Using a cyclooxygenase-2 overexpressing mouse, the authors report the first example of an endogenously generated profile of specific lipid-peroxidation-derived DNA adducts in mammalian tissues.

16. Liu X, Lovell MA, Lynn BC: **Detection and quantification of endogenous cyclic DNA adducts derived from trans-4-hydroxy-2-nonenal in human brain tissue by isotope dilution capillary liquid chromatography nanoelectrospray tandem mass spectrometry**. *Chem Res Toxicol* 2006, **19**:710-718.

17. Pluskota-Karwatka D, Pawlowicz AJ, Kronberg L: **Formation of malonaldehyde-acetaldehyde conjugate adducts in calf thymus DNA**. *Chem Res Toxicol* 2006, **19**:921-926.

18. Cheng G, Shi Y, Sturla SJ, Jalas JR, McIntee EJ, Villalta PW, Wang M, Hecht SS: **Reactions of formaldehyde plus acetaldehyde with deoxyguanosine and DNA: formation of cyclic deoxyguanosine adducts and formaldehyde cross-links**. *Chem Res Toxicol* 2003, **16**:145-152.

19. Nair DT, Johnson RE, Prakash L, Prakash S, Aggarwal AK: **Hoogsteen base pair formation promotes synthesis opposite the 1,N^6-ethenodeoxyadenosine lesion by human DNA polymerase ι**. *Nat Struct Mol Biol* 2006, **13**:619-625.

20. Choi JY, Zang H, Angel KC, Kozekov ID, Goodenough AK, Rizzo CJ, Guengerich FP: **Translesion synthesis across 1,N^2-ethenoguanine by human DNA polymerases**. *Chem Res Toxicol* 2006, **19**:879-886.

21. Mishina Y, Yang C, He C: **Direct repair of the exocyclic DNA adduct 1,N^6-ethenoadenine by the DNA repair AlkB proteins**. *J Am Chem Soc* 2005, **127**:14594-14595.

22. Lin CH, Leow HT, Huang SC, Nakamura J, Swenberg JA, Lin PH: **Induction of cytotoxicity, aldehydic DNA lesions, and poly(ADP-ribose) polymerase-1 activation by catechol derivatives of pentachlorophenol in calf thymus DNA and in human breast cancer cells**. *Chem Res Toxicol* 2005, **18**:257-264.

23. Tozlovanu M, Faucet-Marquis V, Pfohl-Leszkowicz A, Manderville RA: **Genotoxicity of the hydroquinone metabolite of ochratoxin A: structure-activity relationships for covalent DNA adduction**. *Chem Res Toxicol* 2006, **19**:1241-1247.

24. Dai J, Sloat AL, Wright MW, Manderville RA: **Role of phenoxyl radicals in DNA adduction by chlorophenol xenobiotics following peroxidase activation**. *Chem Res Toxicol* 2005, **18**:771-779.

25. Faucet V, Pfohl-Leszkowicz A, Dai J, Castegnaro M, Manderville RA: **Evidence for covalent DNA adduction by ochratoxin A following chronic exposure to rat and subacute exposure to pig**. *Chem Res Toxicol* 2004, **17**:1289-1296.

26. Dai J, Wright MW, Manderville RA: **An oxygen-bonded C8-deoxyguanosine nucleoside adduct of pentachlorophenol by peroxidase activation: evidence for ambident c8 reactivity by phenoxyl radicals**. *Chem Res Toxicol* 2003, **16**:817-821.

27. Pfohl-Leszkowicz A, Manderville RA: **Ochratoxin A: an overview on toxicity and carcinogenicity in animals and humans**. *Mol Nutr Food Res* 2007, **51**:61-99.

28. •• Vaidyanathan VG, Villalta PW, Sturla SJ: **Nucleobase-dependent reactivity of a quinone metabolite of pentachlorophenol.** *Chem Res Toxicol* 2007, in press.
Structurally diverse adducts generated from reactions of a quinone metabolite of PCP are identified.

29. Gold B, Marky LM, Stone MP, Williams LD: **A review of the role of the sequence-dependent electrostatic landscape in DNA alkylation patterns**. *Chem Res Toxicol* 2006, **19**:1402-1414.

30. Tichenor MS, Trzupek JD, Kastrinsky DB, Shiga F, Hwang I, Boger DL: **Asymmetric total synthesis of (+)- and ent-(−)-yatakemycin and duocarmycin SA: evaluation of yatakemycin key partial structures and its unnatural enantiomer**. *J Am Chem Soc* 2006, **128**:15683-15696.

31. Tietze LF, Krewer B, Frauendorf H, Major F, Schuberth I: **Investigation of reactivity and selectivity of DNA-alkylating duocarmycin analogues by high-resolution mass spectrometry**. *Angew Chem Int Ed Engl* 2006, **45**:6570-6574.

32. • Trzupek JD, Gottesfeld JM, Boger DL: **Alkylation of duplex DNA in nucleosome core particles by duocarmycin SA and yatakemycin**. *Nat Chem Biol* 2006, **2**:79-82.
In this study, the question of sequence-dependent DNA alkylation, addressed in several of the preceding references, is examined in relation to nuclear DNA structure.

33. Gong J, Neels JF, Yu X, Kensler TW, Peterson LA, Sturla SJ: **Investigating the role of stereochemistry in the activity of anticancer acylfulvenes: synthesis, reductase-mediated bioactivation, and cellular toxicity**. *J Med Chem* 2006, **49**:2593-2599.

34. Zurbig P, Renfrow MB, Schiffer E, Novak J, Walden M, Wittke S, Just I, Pelzing M, Neususs C, Theodorescu D *et al.*: **Biomarker discovery by CE-MS enables sequence analysis via MS/MS with platform-independent separation**. *Electrophoresis* 2006, **27**:2111-2125.

35. Ohnesorge J, Neususs C, Watzig H: **Quantitation in capillary electrophoresis-mass spectrometry**. *Electrophoresis* 2005, **26**:3973-3987.

36. Feng YL, Zhu J: **On-line enhancement technique for the analysis of nucleotides using capillary zone electrophoresis/mass spectrometry**. *Anal Chem* 2006, **78**:6608-6613.

37. Brown K, Tompkins EM, White IN: **Applications of accelerator mass spectrometry for pharmacological and toxicological research**. *Mass Spectrom Rev* 2006, **25**:127-145.

38. Tompkins EM, Farmer PB, Lamb JH, Jukes R, Dingley K, Ubick E, Turteltaub KW, Martin EA, Brown K: **A novel ^{14}C-postlabeling assay using accelerator mass spectrometry for the detection of O6-methyldeoxy-guanosine adducts**. *Rapid Commun Mass Spectrom* 2006, **20**:883-891.

39. Gong J, Sturla SJ: **A synthetic nucleoside probe that discerns a DNA adduct from unmodified DNA**. *J Am Chem Soc* 2007, **129**:4882-4883.

40. •• Kanaly RA, Hanaoka T, Sugimura H, Toda H, Matsui S, Matsuda T: **Development of the adductome approach to detect DNA damage in humans**. *Antioxid Redox Signal* 2006, **8**:993-1001.
In this study, a common fragment ion corresponding to loss of deoxyribose is monitored as an indicator of adduct formation. This new method offers the potential for rapidly evaluating adduct profiles containing previously unknown forms of DNA modification.

41. Feng Y-L, Lian H, Zhu J: **Application of pressure assisted electrokinetic injection technique in the measurements of DNA oligonucleotides and their adducts using capillary electrophoresis-mass spectrometry**. *J Chromat A* 2007, **1148**:244-249.