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Analysis of DNA adducts using high-performance separation techniques coupled to electrospray ionization mass spectrometry

Christine L. Andrews, Paul Vouros, Andreas Harsch*

Department of Chemistry and the Barnett Institute, Northeastern University, 341 Mugar Building, 360 Huntington Avenue, Boston, MA 02115, USA

Abstract

Identification and quantitation of covalent carcinogen–DNA adducts, an important class of biomarkers, is an integral goal in toxicological research. Since these adducts are commonly present at very low levels in in vivo samples, sensitive and specific analytical methodologies are imperative for accurate detection, characterization and quantitation. High-performance separations coupled to electrospray mass spectrometry (ESI-MS) provide the sensitivity and specificity required for the analysis of DNA adducts. This review provides an overview over the research conducted in this area, focusing on the application of HPLC–ESI-MS and CE–ESI-MS techniques for structural analysis and quantitation of modified nucleosides, nucleotides and oligonucleotides.

Keywords: Mass spectrometry; DNA adducts; Nucleosides; Nucleotides; Oligonucleotides

1. Introduction

Mutations can be induced by a wide variety of chemical species, mainly through initial covalent binding of electrophilic or radical intermediates to DNA [1,2]. Covalent binding to DNA, herein referred to as DNA adduction, leads to structural modification of nucleic acid constituents. Normally, chemical damage to genetic material is promptly repaired by cellular defense systems; however, a small fraction of primary damage events leads to permanent mutations, which in turn can trigger the development of degenerative processes such as cancer. Historically, cancer risk assessment strategies have relied on measuring external exposure to carcinogens. Direct analysis of DNA adducts has proven to be more accurate and reliable in determining the carcinogenicity of xenobiotic compounds as well as allowing investigation of endogenous carcinogens [3]. This methodology allows direct quantitation of the primary damage to genetic material and therefore can account for varying bioavailabilities as well as cellular detoxification mechanisms [4].

A number of analytical methodologies have been developed to detect, identify and quantitate DNA adducts. Experimental approaches generally involve isolation and digestion of target DNA after exposure to a carcinogen, yielding a complex mixture of nucleic acid fragments.

Investigation of DNA adducts requires detection of about one adduct in 10^6–10^8 unmodified nucleobases in individuals who have not suffered from unusual exposure [5,6]. Since modifications are present at such low levels, detection of DNA adducts requires analytical techniques that are highly sensitive and specific. Analysis of physiological samples

*Corresponding author. Fax: +1-617-373-2693.
E-mail address: aharsch@lynx.neu.edu (A. Harsch)

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presents additional challenges due to limited access to tissue or blood. For example, one gram of wet tissue typically contains ca. 1 mg of DNA; 1 ml of blood contains ca. 40 μg of DNA. Assuming 1 modification in 10^6 bases in 1 mg of DNA and a recovery of 10%, the yield of adducted constituents will be approximately 75 pg (ca. 150 fmol).

Experimental approaches such as ^{32}P-postlabeling [7], immunoassays [8], and laser-induced fluorescence (LIF) detection [9–11] permit detection of DNA adducts at levels ranging from one adduct in 10^7 to 10^{10} bases, but have several limitations. Analysis by ^{32}P-postlabeling is very sensitive, but entails use of radioactive materials, and can produce ambiguous results due to nonspecific labeling. Similar complications may also arise using LIF detection. This technique relies on tagging the desired products with a fluorophore prior to analysis. Nonspecifically labeled products can also be formed in this derivatization step, and lead to the observation of false positives. Highly specific detection can be accomplished using immunoassays, however, synthesis of epitopes and specific antibodies is time consuming and costly, and the additional sample handling may cause critical sample loss. Analysis by immunoassays is also sensitive to false positives due to cross-reactivity with structurally similar adducts. In addition, all aforementioned techniques do not permit de novo structural identification and have to rely on comparison with authentic standards.

Over the past decade, mass spectrometry (MS) has gained tremendous popularity for the detection of biomolecules. Novel soft ionization techniques such as matrix-assisted laser desorption ionization (MALDI) [12–14] and electrospray ionization (ESI) [15,16] have enabled routine analysis of non-volatile and fragile molecules by MS. Advances in instrument design have dramatically increased the sensitivity of mass spectrometers, allowing detection of attomoles of analyte. Moreover, MS can also provide detailed structural information using collision induced dissociation (CID) [17,18] or post source decay (PSD) [19].

On-line coupling of MS to separation techniques such as high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) can further enhance the capabilities of MS detection. While on-line coupling of CE to MALDI has been demonstrated [20], ESI is currently the most utilized ionization technique for interfacing LC or CE separations to MS.

In this review, analysis of modified DNA constituents using LC–ESI-MS and CE–ESI-MS will be discussed.

2. General considerations

DNA adduct samples for LC–MS or CE–MS analysis are generated synthetically or by isolation and digestion of DNA after exposure to a carcinogen in vitro or in vivo. The digestion procedure determines the chemical nature of the hydrolysate, and thus needs to be tailored to the selected analysis technique. Partial enzymatic digestion of DNA furnishes oligonucleotides of varying lengths, whereas total hydrolysis yields nucleotides, nucleosides, or nucleobases. Since adducts are present in very small concentrations, preconcentration techniques such as liquid–liquid extraction or solid-phase extraction (SPE) can be applied to remove the bulk of unmodified products.

Covalently modified nucleosides and nucleobases are commonly analyzed using LC–MS. Adducts can be separated from unmodified constituents using reversed-phase LC due to differences in hydrophobicity. ESI-MS analysis is performed in positive ion spray mode, which is especially advantageous for applications requiring maximum sensitivity. In contrast, the presence of charged phosphate groups in modified nucleotides and oligonucleotides make these analytes well-suited for MS detection in negative ion spray mode. Analysis of modified nucleotides and oligonucleotides is critical to develop a full understanding of the chemical behavior of a carcinogen since it allows detection of structurally diverse adducts, i.e., adducts containing covalent modifications on the phosphate moiety. Furthermore, MS and MS–MS analysis of modified oligonucleotides can reveal the position of modification within a given sequence.

3. High-performance liquid chromatography coupled to electrospray ionization mass spectrometry

As stated above, nucleoside adducts are generally

analyzed by HPLC–MS using positive electrospray ionization. Early work in this field utilized capillary LC coupled to continuous flow fast atom bombardment mass spectrometry (CF-FAB-MS) and demonstrated the utility of this approach for the detection and characterization of both targeted and unknown adducts [21]. The first example of LC–ESI-MS analysis of nucleoside adducts was published in 1995 by Chaudhary et al. [22]. A list of references for the analysis of DNA adducts by HPLC–ESI-MS is provided in Table 1. In prior work, the researchers had analyzed pentafluorobenzyl derivatized malondialdehyde–deoxyguanosine adducts by gas chromatography–electron capture negative chemical ioniza-

Table 1
HPLC–MS analyses of different classes of deoxynucleic acid adducts

Ref.	HPLC–MS	
	Analytes	MS detection mode
Modified nucleosides		
[22]	Pyrimido[1,2-*a*]purine-10(3H)-one-2′-deoxyribose	CNL
[24]	N^6-Oxopropenyl-2′-deoxyadenosine	CNL
[25]	2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine 2′-deoxyguanosine	SRM
[26]	7-(2-Oxopropyl)-1,N^2-etheno 2′-deoxyguanosine	SIM, SRM
[30]	1,N^2-Etheno(ϵ)guanosine, 5,6,7,9-Tetrahydro-7-hydroxy-9-oxoimidazo[1,2-*a*]purine deoxyribose	SRM
[37]	Malondialdehyde, crotonaldehyde, 2-hexenal and 4-hydroxy-2-nonenal modified deoxyguanosine and deoxyadenosine	CNL, MRM
[34]	8-Hydroxy-deoxyguanosine	SRM
[35]	8-Oxo-7,8-dihydro-2′-deoxyguanosine	SIM, SRM
Modified nucleobases		
[27]	N^2,3-Ethenoguanine	Full scan, SIM
[28]	1,N^6-Etheno-adenine	Full scan, SIM, CID
[29]	7-(2-Hydroxyethyl)guanine	Full scan, SIM
[30]	5,6,7,9-Tetrahydro-7-hydroxy-9-oxoimidazo[1,2-*a*]purine, 1,N^2-Etheno(e)guanine	SRM
[31]	1,2-Epoxybutene adducts of adenine and guanine	CID
[32]	*N*-7-(2-Hydroxy-3-buten-1-yl)guanine	SRM
[33]	*S*-[2-(N^7-Guanyl)ethyl]glutathione	SIM, SRM
[38]	*N*-(Deoxyguanosin-8-yl)-4-aminobiphenyl	SIM, in source CID
Modified nucleotides		
[37]	Malondialdehyde, crotonaldehyde, 2-hexenal and 4-hydroxy-2-nonenal modified deoxyguanosine monophosphate and adenosine monophosphate	CNL, MRM
[39]	Bisphosphonate drug deoxynucleotide metabolites	CID
[40]	Cyclic deoxynucleotides	CID, MRM
[41]	2,2-Bis[4-(2,3-epoxypropoxy)phenyl]propane Bisphenol A diglycidyl ether modified deoxynucleotides	CID, SIM, SRM
Modified oligonucleotides		
[42]	Unmodified oligonucleotides	Full scan
[43]	Unmodified polythymidines and synthetic plasmid oligonucleotides, Phosphorothioate oligonucleotides	Full scan
[44]	Phosphorothioate oligonucleotides	Full scan
[45]	Phosphorothioate oligonucleotides	CID

CNL=Constant neutral loss.
SRM=Selected reaction monitoring.
SIM=Single ion monitoring.
MRM=Multiple reaction monitoring.
CID=Collision induced dissociation.

tion mass spectrometry (GC–EC-NCI-MS) [23]. In order to circumvent derivatization procedures for GC–MS analysis, an LC–MS based approach was subsequently chosen, as shown in Fig. 1 [22]. Acquired MS and MS–MS data provided detailed structural information, which verified earlier findings and led to the identification of a hitherto unknown adduct at adenine residues [24]. This methodology was readily amenable to quantitative analysis of DNA adducts using selected reaction monitoring (SRM). SRM scanning traces the intensity of a single transition in a CID experiment, allowing sensitive and selective detection [17]. This work demonstrated for the first time the utility of on-line LC–ESI-MS and LC–ESI-MS–MS for DNA adduct characterization and quantitation.

Concurrently, Ringden et al. published a study on qualitative and quantitative analysis of nucleosides bearing heterocyclic aromatic amine (HAA) modifications. The researchers reported detection of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine–deoxyguanosine adducts by LC–MS–MS at picogram levels using SRM scanning [25]. Additionally, the LC–MS methodology was also compared to parallel studies by ^{32}P-postlabeling. The results obtained by both techniques were in qualitative agreement, and mass spectrometry allowed identification of a new adduct which could not be characterized by ^{32}P-postlabeling experiments.

Liu et al. used LC–ESI-MS and LC–ESI-MS–MS to investigate 7-(2-oxopropyl)-1,N^2-etheno dG adducts, formed in the reaction of bioactivated *N*-nitrosopiperidine with DNA [26]. Identification of the target adduct and its hemiaminal precursors proved particularly useful since etheno adducts have also been linked to carcinogenesis induced by vinyl chloride and related compounds.

Damage to DNA induced by alkylating agents such as epoxides or alkyl halides also was investigated using LC–MS. Alkylation of DNA may induce depurination yielding free modified nucleobases. After exposure to these modifying reagents, acid

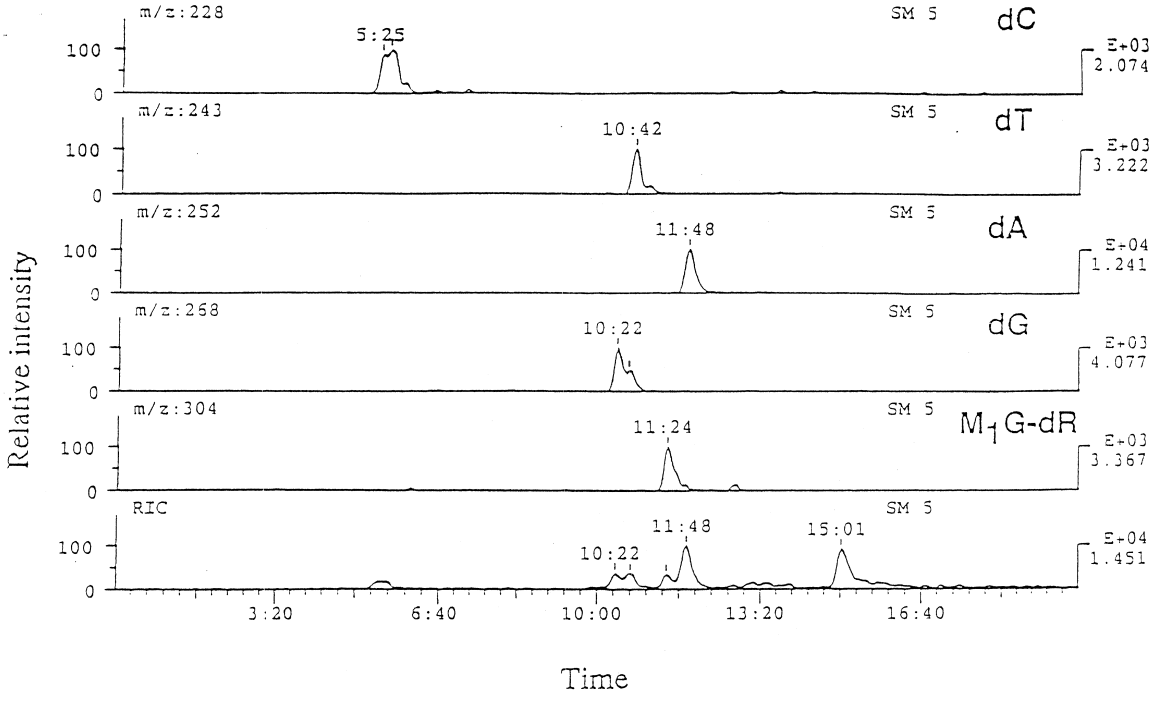

Fig. 1. LC–ESI-MS–MS analysis of the hydrolysate from sodium malondialdehyde (NaMDA)-modified calf thymus DNA. The selected mass data traces of *m*/*z* 228, 243, 252, 268 and 304 correspond to the MH^+ ions of dC, dT, dA, dG and the targeted adduct (M_1G-dR), respectively. The peak at 11:24 is due to the targeted adduct M_1G-dR. (Reprinted from Chaudhary et al., J. Mass Spectrom. 30 (1995) 1157–1160. Copyright 1995 Wiley–Interscience.)

hydrolysis is normally employed to generate a mixture of modified and unmodified nucleobases. In this case, total degradation of the DNA is advantageous, since it ensures absence of nucleoside or nucleotide impurities. Yen et al. carried out quantitative analysis of guanine adducts formed in in vitro and in vivo reactions of DNA with chloroethylene oxide [27]. A subsequent study disclosed a new methodology allowing quantitation of 1,N^6-ethenoadenine in rat urine [28]. Off-line sample preparation using immunoassays allowed specific separation of the adducted nucleobases from unmodified constituents and the biological matrix. LC–MS analysis of 7-(2-hydroxyethyl)guanine, formed when DNA is exposed to ethylene oxide has been published by Leclercq et al. [29]. In a related investigation, Müller et al. obtained slightly higher sensitivity when adducted nucleosides rather than nucleobases were analyzed [30]. This publication also provided a comparison of a series of adducts from the exposure of DNA to vinyl chloride in vitro.

Kambouris et al. studied 1,2-epoxybutene (BDO) adducts in vitro [31]. LC–MS–MS analysis allowed separation and detection of eight BDO–guanine and seven BDO–adenine adducts. In a related line of work, Tretyakova et al. isolated 1,3-butadiene epoxide adducts from in vitro and in vivo sources [32]. LC–MS analysis revealed different adduct profiles in the in vitro and in vivo samples. Unambiguous identification of isomeric adducts was accomplished using on-line MS–MS analysis, yielding characteristic fragmentation patterns. Adducts were detected at levels less than one modification in 10^5 bases using single ion monitoring (SIM) and SRM. These scanning modes are especially useful in the analysis of complex reaction mixtures, since they allow specific tracing of the target compounds.

An interesting nucleobase conjugate was investigated by Huang et al. in 1998 [33]. The researchers used LC–MS and LC–MS–MS to analyze *S*-[2-N^7-guanyl)ethyl]glutathione. This adduct is formed by modification of DNA with 1,2-dihaloethanes. These alkylating agents are common water pollutants from industrial sources, and are used as gasoline additives and intermediates in the production of halogenated organics. They are bioactivated by conjugation with glutathione and subsequently attack DNA at guanine residues. Tandem mass spectrometry allowed verification of the proposed structure and lowered detection limit from 100 pg to 5 pg on-column using SRM detection.

Monitoring of oxidative damage to DNA has been performed by LC–MS analysis [34,35]. This approach has proven to be more versatile than HPLC separation with electrochemical detection due to the universal detection capability of MS. It is also more accurate than GC–MS based methodology, since analysis of oxidative DNA metabolites by GC–EC-NCI-MS is known to produce artificially high concentrations of the derivatized adducts. The elevated levels can be attributed to oxidation processes during sample preparation [36]. Since LC–MS does not require sample derivatization, artifacts due to extracellular oxidation are much less prominent. Due to these advantages, this approach has become a standard method for sensitive and accurate detection of oxidized DNA constituents.

A comparison of DNA adduct detection using on-line liquid chromatography coupled to different mass spectrometers has been published by Doerge et al. [37]. Single quadrupole MS was useful for molecular mass determination and in-source CID studies, whereas a triple quadrupole instrument demonstrated higher sensitivity and selectivity. The researchers determined a hybrid quadrupole orthogonal-acceleration time-of-flight instrument provided the highest sensitivity and allowed studies on minimal sample quantities. In a related line of work, Beland et al. carried out quantitative measurements on *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl, a known aminobiphenyl–dG adduct [38]. Quantitation was performed by LC–MS, dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA) and ^{32}P-postlabeling. It was concluded that quantitation by HPLC–ESI-MS using SIM and calibration curves obtained from deuterium labeled analogs was most consistent with independent results obtained by ^{3}H incorporation.

Numerous examples of modified nucleotides and oligonucleotides analyzed by on-line LC–ESI-MS also have been reported. Since these analytes contain negatively charged phosphate groups, they are commonly transferred into the gas phase using negative electrospray ionization [37]. Negative electrospray requires careful adjustment of sample conditions and instrument tuning to obtain sensitivity comparable to

positive electrospray ionization. However, these restrictions are outweighed by the benefits of enabling analysis of structurally diverse adducts. Auriola et al. analyzed nucleotide analogues of bisphosphonate drugs using negative electrospray ionization, since these adducts were not amenable to enzymatic hydrolysis [39]. Drug metabolites were readily identified due to the characteristic gas-phase fragmentation patterns of the bisphosphonate moiety. Witters et al. published an investigation of cyclic nucleotides using LC–ESI-MS and LC–ESI-MS–MS [40]. MS–MS data revealed the cyclization patterns of phosphate groups. This study also provided insight into the dependence of MS signal intensity on the pH of the spray buffer. In negative electrospray ionization, decreased signal intensity was observed at high pH values due to competition between negatively charged analyte ions and acetate ions in the mobile phase buffer. Signal intensity was also compromised at low pH, since protonation of the phosphate groups decreased the overall negative charge density of the analytes.

In an attempt to increase MS detection sensitivity in the analysis of guanosine adducts with bisphenol diglycidyl ether, Vanhoutte et al. designed a nanoflow LC–nanospray ESI-MS system [41]. The system increased mass sensitivity by a factor of 3300 when compared to normal bore LC–ESI-MS, due to lower flow-rates, increased spray efficiency, and more uniform droplet formation.

In recent years, analysis of oligonucleotides by LC–ESI-MS and LC–ESI-MS–MS has generated a great deal of interest. Groundbreaking research in this area was carried out by Bleicher and Bayer, who investigated a series of solvent systems for on-line HPLC–ESI-MS analysis of oligonucleotides [42]. The researchers emphasized the need for solvent systems supporting efficient separation and ionization. Unfortunately, these requirements may not necessarily be mutually inclusive. Triethylammonium acetate (TEAA) is often added as an ion-pairing agent in reversed-phase LC separations of oligonucleotides. It has been observed, however, that this additive drastically reduces the ion production in electrospray ionization. This dilemma leads to a compromise in either separation performance or detection sensitivity. In the work by Bleicher and Bayer, improved MS detection was obtained without loss of LC separation when ammonium acetate and acetonitrile were used as the solvent system.

In an effort to further overcome the above limitations, Apffel et al. developed a novel solvent system for LC–MS analysis of oligonucleotides containing 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)–triethylamine (TEA) additives [43]. This methodology yielded comparable separation results to the TEAA based solvent system, but afforded superior ESI-MS sensitivity. Optimization of the HFIP and TEA concentrations yielded a two-fold increase in the signal intensity (compared to TEA alone) and showed no loss in chromatographic resolution (compared to TEAA). Baseline separation of a series of oligonucleotides ranging in length from a 15-mer to a 75-mer was obtained, see Fig. 2. The success of the HFIP–TEA solvent system is due in large part to the high volatility of the HFIP component, which leads to a decreased competition with analyte ions for ionization.

Gaus et al. [44] and Griffey et al. [45] have provided examples for LC–ESI-MS analysis of modified oligonucleotides. The research focused on the analysis of phosphorothioate oligonucleotides (PS ODN), a novel class of antisense therapeutics. LC–MS analysis of 2′-deoxyphosphorothioate oligonucleotides and their in vivo metabolites has been carried out using tripropylamine (TPA) in 2-propanol–water [44]. In related work, liquid chromatography coupled to electrospray ion trap mass spectrometry (LC–ESI-ITMS) was used to study the metabolism of a PS ODN drug extracted from pig kidney samples [45]. This study used the HFIP–TEA solvent system designed by Apffel and associates (vide supra). MS and MS–MS detection of the analytes allowed identification of all known metabolites.

4. Capillary electrophoresis coupled to electrospray ionization mass spectrometry

Since its inception in 1981, CE [46] has gained tremendous popularity for high efficiency separations, and has been widely used in the field of bioanalytical chemistry. On-line interfacing of CE to ESI-MS was reported by Olivares et al. in 1987 [47]. Over the past decade, alternative interface designs

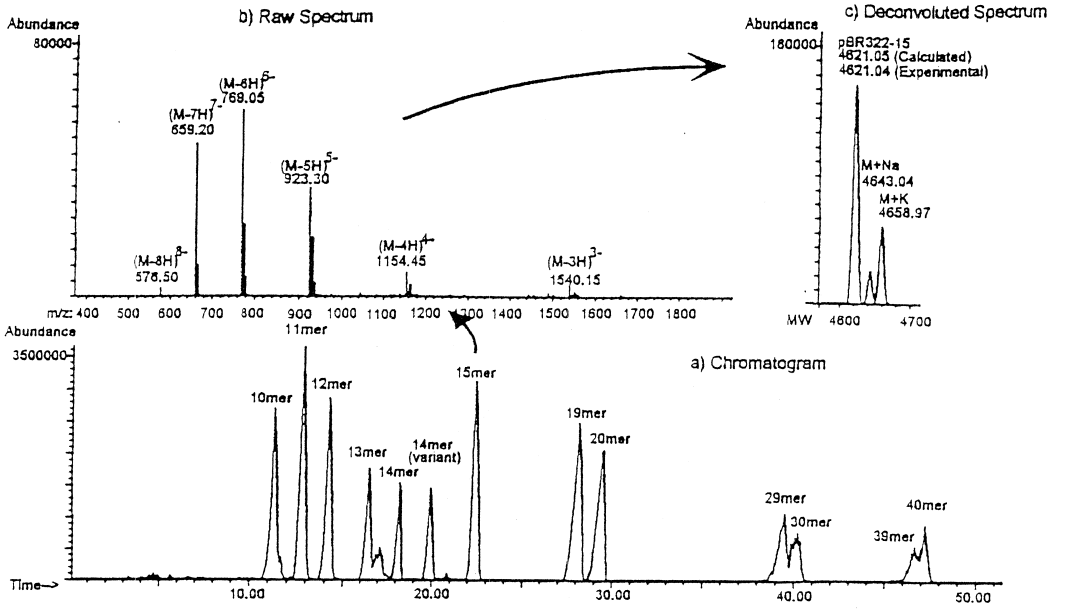

Fig. 2. Analysis of a mixture of oligonucleotides by HPLC–ESI-MS. (a) Total ion chromatogram, (b) raw mass spectrum, (c) deconvoluted mass spectrum. (Reprinted from Apffel et al., Anal. Chem. 69 (1997) 1320–1325. Copyright 1997 American Chemical Society.)

have been introduced [48,49]. A series of excellent reviews have been published on the fundamentals and applications of CE–MS [50–53]. The first application of CE–ESI-MS analysis to DNA adducts was demonstrated by Janning et al. [54]. References for CE–ESI-MS analysis of DNA adducts are listed in Table 2. Styrene oxide-modified DNA was subjected to partial enzymatic digestion yielding a complex mixture of modified and unmodified oligonucleotides. Capillary zone electrophoresis (CZE) provided baseline separation into groups of oligonucleotides of varying lengths due to pronounced differences in electrophoretic mobilities. Presence of covalently modified oligonucleotide analytes was verified by full scan MS analysis (Fig. 3). In subsequent work, on-line preconcentration was employed to enhance the sensitivity of the assay [55]. Results indicated that styrene oxide did not exhibit sequence specificity, but showed strong preference for purines versus pyrimidines in in vitro experiments [56].

A major drawback of CE is its low mass and volume capacity in terms of sample introduction. The injection volume in CE is usually insufficient for adequate concentration detection limits of biological samples. This limitation can be overcome by off-line or on-line preconcentration methodologies. Sample stacking was first introduced by Chien and Burgi [57] and allows preconcentration of the analytes in the capillary, resulting in sensitivity gains of several orders of magnitude. Schrader et al. [55,56] used on-line sample stacking to increase the amount of sample injected, thus boosting the sensitivity of the CE–MS system without overloading the capillary and causing precipitation of the analyte. This technique was investigated systematically in the analysis of nucleotide and oligonucleotide adducts by Wolf and Vouros [58]. The authors reported a 100-fold increase in sensitivity using sample stacking for the determination of *N*-acetyl-2-aminofluorene–dGMP adducts by CZE–CF-FAB-MS at a detection limit of $6.3 \cdot 10^{-8}$ *M*. A combination of off-line (SPE) and on-line preconcentration methodologies enabled sensitive detection of benzo[*a*]pyrene–dGMP adducts (four adducts in 10^7 unmodified bases) by CZE–ESI-MS [59]. A significant limitation of sample stacking is the requirement of a well-defined solvent system. Consequently, sample stacking provides only minimal improvements when used for samples in biological matrices.

Table 2
CE–MS analyses of different classes of deoxynucleic acid adducts

Ref.	CE–MS	
	Analytes	MS detection mode
Modified oligonucleotides		
[54]	Styrene oxide modified oligonucleotides	Full scan
[55]	Styrene oxide modified oligonucleotides	Full scan
[66]	alkylated oligonucleotides and oligonucleotides containing halogenated nucleobases	Full scan
[67]	*N*-(Acetylamino)fluorene modified oligonucleotides	Full scan, CID
[71]	Unmodified oligonucleotides	CID
Modified nucleotides		
[55]	N^7, O-6, N^2 modified guanosine monophosphate	Full scan
[56]	N^7, O-6, N^2 modified guanosine monophosphate and adenosine monophosphate	Full scan
[59]	*anti*-7,8,9,10-Tetrahydroxybenzo[*a*]pyrene-7,8-diol-9,10-epoxide modified nucleotides	CID, SRM
[60]	Phenyl glycidyl ether modified nucleotides	Full scan, CID
[61]	Phenyl glycidyl ether 5′ phosphate modified nucleotides	CID
[62]	Phenyl glycidyl ether 5′ phosphate modified nucleotides	CID
[63]	Unmodified nucleotides, NAD^+ and FAD^+ dinucleotides	CID
Modified nucleosides		
[69]	Benzo[*g*]chrysene and 5,6-dimethylchrysene modified deoxypurines	Full scan
[70]	Styrene oxide modified adenosine adducts	CID

CID=Collision induced dissociation.
SRM=Selected reaction monitoring.

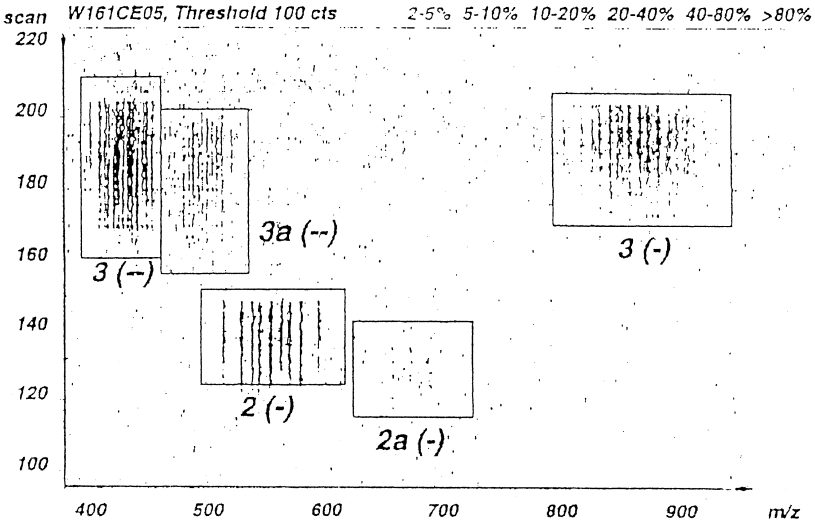

Fig. 3. Two-dimensional plot of the CZE–MS analysis of styrene oxide-modified and unmodified oligonucleotides. Numbers indicate the length of the oligonucleotides, charge states are indicated with (-) or (- -), and adducts are labeled ''a''. (Reprinted from Schrader et al., Arch. Toxicol. 71 (1997) 588–595. Copyright 1997 Springer-Verlag.)

Deforce and co-workers published a detailed study of 2′-deoxynucleotides bearing phenyl glycidyl ether (PGE) modifications [60,61]. CZE–ESI-MS and CZE–ESI-MS–MS, in conjunction with sample stacking, allowed separation and identification of a series of mono- and dialkylated dG derivatives. Furthermore, an adduct stemming from alkylation of the phosphate moiety was also detected. As shown in Fig. 4, additional phosphate-alkylated adducts were detected in a subsequent study, which also determined the presence of a hydrolytic deamination product (dUMP) caused by the alkylation of dCMP [62].

Zhao et al. employed capillary isotachophoresis (cITP) in conjuction with CZE–ESI-MS analysis for the detection of unmodified mono- and dinucleotides [63]. The initial cITP step focused the analytes into discrete, sharp bands on-column, and subsequent CZE–MS analysis provided molecular mass and structural information of the nucleotides and dinucleotides. cITP–CE–UV with a polyethylene glycol (PEG) matrix had previously been used by Auriola et al. for the baseline separation of oligonucleotides ranging in length from 19- to 24-mers, with a detection limit of 100 ng/ml [64]. This method allowed injection of a 20× larger sample volume without loss of resolution.

The selectivity of CE separations can be dramatically increased using buffer additives. In DNA adduct research, CE buffer systems containing a water soluble polymer additive, polyvinylpyrrolidone (PVP), have been employed to accomplish separations of isomeric and structurally related analytes. PVP acts as a pseudophase in CE separations, allowing resolution on the basis of differences in hydrogen bonding, dipole–dipole interactions, and/or hydrophobic interactions [65]. Barry et al. exploited this concept for the analysis of short modified and unmodified oligonucleotides [66]. CE(PVP)–ESI-MS analysis of oligonucleotides differing only in minor hydrophobic modifications yielded baseline separation of all analytes. This methodology was also used to analyze a series of oligonucleotides containing covalent (*N*-acetylamino)fluorene (AAF) modifications [67]. Separation was accomplished for isomeric AAF-modified hexanucleotides differing only in their base sequences, Fig. 5. On-line MS–MS detection allowed unambiguous identification of the

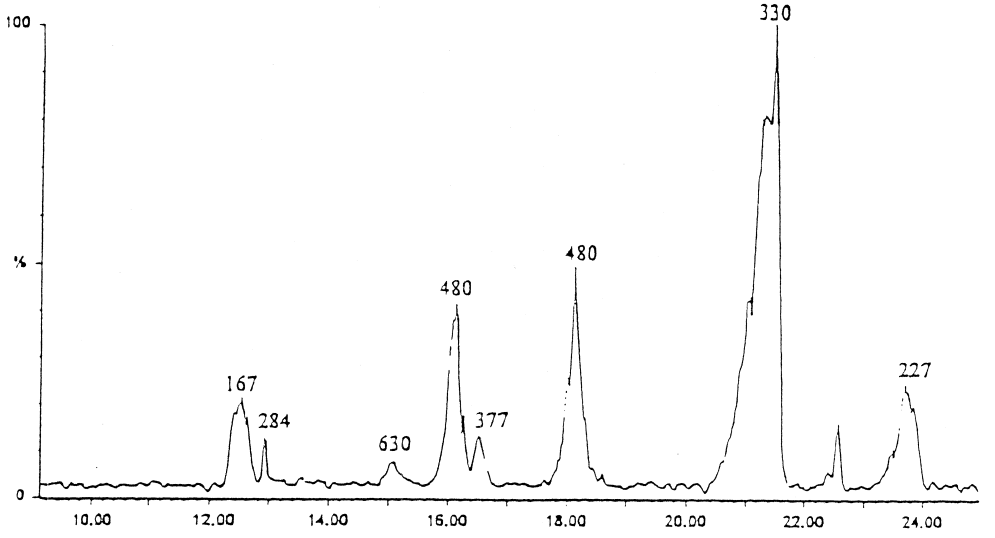

Fig. 4. CE–MS of the reaction mixture of dAMP with phenyl glycidyl ether (PGE) using full-scan MS detection. Detected signals in order of increasing migration time: *m/z* 167, PGE-diol; *m/z* 284, adduct of adenine with PGE; *m/z* 630, dialkylated dAMP; *m/z* 480, dAMP alkylated on the 5′-phosphate moiety; *m/z* 377, deoxyribose methoxylated on C-1′ and phosphorylated on C-5′ with PGE adduct on the phosphate moiety; *m/z* 480, dAMP alkylated on the heterocyclic moiety; *m/z* 330, unmodified dAMP; and *m/z* 227, deoyribose methoxylated on the C-1′ and phosphorylated on C-5′. (Reprinted from Deforce et al., Anal. Chem. 68 (1996) 3575–3584. Copyright 1996 American Chemical Society.)

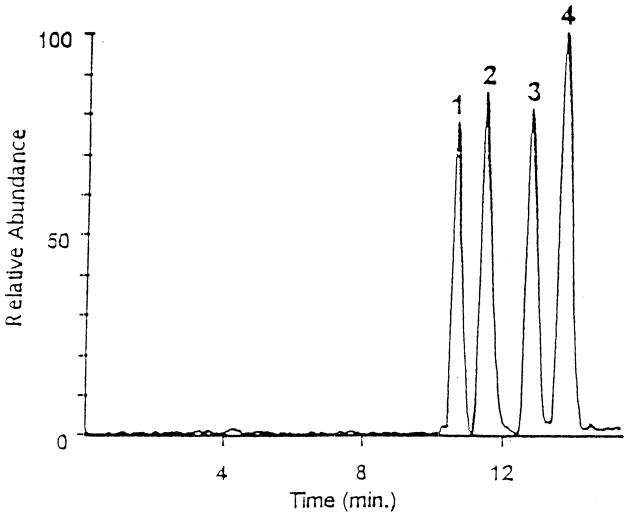

Fig. 5. CE(PVP)–ESI-MS analysis of four isomeric, acetylaminofluorene-modified oligonucleotides in order of migration: ATG*CTA, ATTCAG*, TG*TAAC, CATG*AT. MS detection of m/z 1005. (Reprinted from Harsch et al., Anal. Chem. 70 (1998) 1320–1327. Copyright 1998 American Chemical Society.)

isomeric analytes based on differences in their gas-phase fragmentation patterns.

Capillary electrochromatography (CEC) coupled to ESI-MS has also been employed for the analysis of DNA adducts. The concept of CEC was originally introduced by Pretorius in 1974 as a hybrid technique between HPLC and open-tubular electrophoresis [68]. This technique exploits the advantages of both separation methodologies, and allows rapid separation of charged and neutral species with high efficiency and selectivity. In DNA adduct research, CEC–ESI-MS has been employed to separate nucleosides adducts of polyaromatic hydrocarbons (PAHs) [69] and styrene oxide [70].

5. Future directions

It now widely accepted that high-performance separation techniques coupled to ESI-MS are powerful tools for the detection and identification of DNA adducts. The effectiveness of HPLC–ESI-MS and CE–ESI-MS will be further enhanced through combination with complementary bioanalytical techniques such as immunoassay and ^{32}P-postlabeling. Alliances such as these will capitalize on the benefits of each methodology, and may therefore further improve both sensitivity and specificity of these assays. Preliminary work in this area has been published by Yen et al. [28] and Beland et al. [38] (vide supra).

Improvements in both separation technologies and mass spectrometry are expected to narrow the gap in sensitivity between ESI-MS detection and ^{32}P-post-labeling. More importantly, the advent of chip-based technologies for sample preparation and separation may revolutionize the analysis of DNA adducts. Progress in this area will enable fully integrated and automated analysis of complex reaction mixtures, minimizing sample losses and increasing the sample throughput. Parallel advances in nanospray ionization will lead to more effective coupling of mass spectrometry to miniaturized separation systems, such as nanoflow capillary LC or chip-based technologies.

Recent advances in MS instrumentation design have also boosted the sensitivity and speed of MS data acquisition. Ion trap mass analyzers are being used preferentially in structural elucidation work due to their excellent MS–MS and MS^n capabilities, and low cost of operation. The latest generation ion trap mass spectrometry (ITMS) systems show further

improved sensitivity and high accuracy of mass detection, making ITMS an attractive choice for structural characterization of DNA adducts. The introduction of hybrid instruments, such as quadrupole-time-of-flight (Q-TOF) mass spectrometers has further expanded the versatility of MS analysis. Detection by ESI-Q-TOF-MS offers high mass resolution and sensitivity. This technology also allows very rapid acquisition of MS data, necessary for reliable detection of sharp chromatographic and electrophoretic peaks. A Q-TOF instrument has been coupled to HPLC for the analysis of DNA adducts by Doerge et al. [37]. Unmodified oligonucleotides 20–120 bases in length have been analyzed by CZE–ESI-Q-TOF-MS [71].

For ultrasensitive detection, Fourier transform (FT) MS instrumentation is now routinely available to a large population of researchers. This technique has not been coupled to separation techniques for the analysis of DNA or its adducts, but has the capability of increasing the sensitivity of LC–MS and CE–MS methodologies. It should also be noted, that accelerator mass spectrometry (AMS) currently achieves levels of detection exceeding ^{32}P postlabeling. However, since this technique does not rely on ESI and cannot provided mass or structural information, it is not discussed further in this review.

In view of these developments it is anticipated that hyphenated techniques such as HPLC–MS and CE–MS will assume a leading role in the analysis of DNA adducts, i.e., in clinical settings and for risk assessment of carcinogen exposure.

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