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Review

Analysis of DNA-bound advanced glycation end-products by LC and mass spectrometry ☆

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Abstract

Sugars and sugar degradation products readily react in vitro with guanine derivatives, resulting in the formation of DNA-bound advanced glycation end-products (DNA-AGEs). The two diastereomers of N^2-(1-carboxyethyl)-2′-deoxyguanosine ($CEdG_{A,B}$) and the cyclic adduct of methylglyoxal and 2′-deoxyguanosine (mdG) (N^2-7-bis(1-hydroxy-2-oxopropyl)-2′-deoxyguanosine have also been detected in cultured cells and/or in vivo. LC–MS/MS methods have been developed to analyze sensitively DNA adducts in vitro and in vivo. In this paper, the chemical structures of possible DNA-AGEs and the application of LC–MS/MS to measure DNA-AGEs are reviewed.
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Keywords: Advanced glycation end-products; N^2-(1-carboxyethyl)-2′-deoxyguanosine; $CEdG_{A,B}$; DNA; LC–MS/MS

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1. Introduction

Carbohydrates are reactive compounds which undergo various types of reactions [1]: the carbonyl group of reducing sugars can bind to nucleophilic partners, such as amines or thiols. Furthermore, elimination and cleavage reactions lead to the formation of sugar degradation products, such as 3-deoxyglucosone, methylglyoxal or glyoxal. Due to their shorter length and/or α-dicarbonyl structure, they are even more reactive than the sugars themselves. In a heated glucose solution, for example, about 70% of the observed protein modifications derive from glucose degradation products, despite of a 1000-fold molar excess of glucose [2]. In vivo, reactive carbonyl compounds are also formed by metabolic pathways [3]. Thus, the presence of reactive carbonyl compounds in vivo – either endogenously formed or administered by nutrition or smoking – has been described as “carbonyl stress”. Reactive carbonyl compounds have adverse effects, for example, by their ability to modify bio-molecules [4]. It has been postulated that reactive carbonyls bind to DNA in a similar way as known for other electrophilic compounds (DNA glycation). Since carbonyl compounds are ubiquitously present in cells, this mechanism would result in a permanent and unavoidable background of DNA glycation products. Similar to other DNA adducts, glycated DNA

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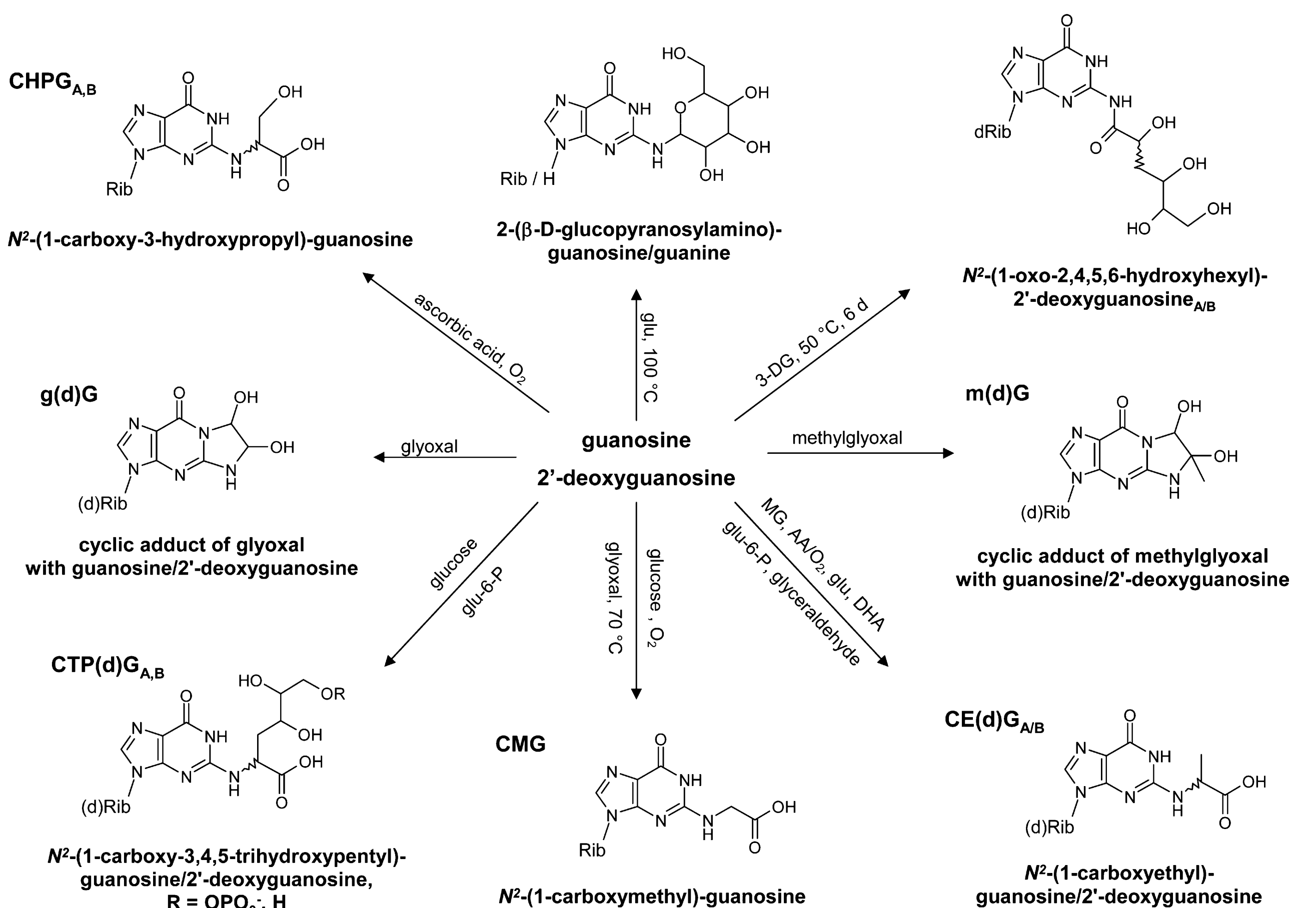

Scheme 1. Structures of guanosine- and 2′-deoxyguanosine-derived AGEs. MG, methylglyoxal; AA, ascorbic acid; glu, glucose; DHA, dihydroxyacetone; 3-DG, 3-deoxyglucosone; glu-6-P, glucose-6-phosphate.

may contribute to the age-related decrease of genomic functionality [5]. Additionally, under conditions of increased carbonyl stress, such as diabetes, kidney dysfunction, or UV irradiation of skin, DNA glycation could be enhanced and thus promote disease related complications [6,7]. DNA glycation may therefore contribute, for example, to the increase of mutations which were observed during aging of transgenic mice [8] or to diabetic embryopathy [9].

2. DNA glycation: from browning to structures

Many years ago, first evidence was obtained that reactive carbonyl compounds can bind to DNA in vitro, leading to the formation of so called DNA-bound advanced glycation end-products (DNA-AGEs). Incubation of DNA with radioactively labeled methylglyoxal, for example, leads to an incorporation of radioactivity into the DNA [10]. Likewise, the incubation of DNA with sugars leads to a characteristic browning [11]. Among the nucleobases, guanine and its derivatives react most easily with the sugars and dicarbonyl compounds [12]. Thus, free guanosine, guanine, or 2′-deoxyguanosine was used in model incubations with sugars and sugar degradation products to identify the structure of possible DNA-adducts. At elevated temperatures, the glucosylamine of 2′-deoxyguanosine and D-glucose was the main product [13], whereas at physiological temperatures, (2*R*,4*S*,5*R*)-2-(N^2-2′-deoxyguanosyl)-4,5,6-trihydroxyhexanoate [N^2-(1-carboxy-3,4,5-trihydroxypentyl)-2′-deoxyguanosine, CTPdG] predominated [14,15] (Scheme 1). In the cell, glucose is mostly phosphorylated to glucose-6-phosphate, so that the latter seems to be a more relevant DNA-AGE precursor in vivo. The reaction of glucose-6-phosphate with 2′-deoxyguanosine resulted in the formation of a CTPdG derivative which was phosphorylated in position 5. The product was indirectly identified by enzymatic conversion into CTPdG [16]. The two diastereomers of 2-[9-(2-deoxy-β-D-ribofuranosyl)-6,9-dihydro-6-oxo-1H-purin-2-ylamino]propionate, also named N^2-(1-carboxyethyl)-2-deoxyguanosine (CEdG$_{A,B}$), were first isolated as reaction products of glyceraldehyde [17]. Later on, it was shown that CEdG or the analogous derivatives from guanosine, guanine or 9-methylguanine – CEG, CEguanine and CEmG – were also formed from a great variety of other sugars and sugar degradation products, such as glucose, ribose, glucose-6-phosphate, dihydroxyacetone, or ascorbic acid [12,16,18,19]. In the presence of oxygen and glucose, lower concentrations of N^2-carboxymethyl-guanosine (CMG) were additionally detectable [20,21].

As observed for protein glycation before, α-dicarbonyl compounds react very efficiently with nucleobases: guanosine, for example, was converted completely into the

cyclic adduct 3-(β-D-erythro-pentafuranosyl)-6,7-dihydro-6,7-dihydroxy-6-methylimidazo[2,3-b]purine-9(8H)one (mG) by a 10-fold excess of methylglyoxal after only 5 min at room temperature [22,23]. However, during extended incubation of 2′-deoxyguanosine with methylglyoxal for several days, the amount of the cyclic adduct seemed to degrade in favor of CEdG [12,24]. Cyclic adducts, analogous to mG, are also formed from glyoxal and guanine derivatives (gdG or gG) [25,26]. In lower concentrations, adducts of methylglyoxal or glyoxal and 2′-deoxyadenosine and 2′-deoxycytidine were identified [24,27].

3. Genotoxic effects of DNA-AGEs

It is well established that reactive carbonyl compounds, such as glyoxal and methylglyoxal, lead to mutations in bacteria and mammalian cells [28,29]. Furthermore, DNA-AGEs could be directly linked to alterations in the DNA structure and functionality. CEdG-adducts, selectively introduced into the DNA, destabilized the *N*-glycosidic bond between CEguanine and the sugar–phosphate DNA backbone, leading to the specific loss of the modified guanine. This process is generally called depurination [30]. Consequently, the occurrence of single-strand breaks was observed in CEdG-modified DNA [31]. The transformation of bacteria cells by glycated plasmids resulted in an increased mutation frequency [31] caused by insertions, deletions, as well as multiple species [32]. Likewise, single-base substitutions and the transposition of an Alu-containing element were observed, when DNA, which was pre-treated with glyoxal or sugars, was transfected into mammalian cells [33,34].

4. Occurrence of DNA-AGEs in vivo

In the meantime, there is evidence that the formation of DNA-AGEs is not only limited to reactions of isolated DNA in vitro, but also takes place under cell culture conditions and in vivo. Using a ^{32}P-postlabelling technique, the cyclic adducts of methylglyoxal and 2′-deoxyguanosine (mdG) have been determined in human lymphocytes or human buccal epithelial cells, which had been exposed to methylglyoxal [29,35,36]. First evidence that CEdG is formed in vivo was obtained by Schneider et al. [37]. Glycated nucleobases were measured in 121 human urine samples using a monoclonal antibody raised against CEdG. The concentrations were between 1.2 and 117 ng CEdG eq/mg creatinine. Later on, the presence of CEdG in human urine was confirmed by immunoaffinity chromatography coupled to HPLC-DAD [38]. Using the same method, CEdG was also detected in the genomic DNA of human smooth muscle cells and bovine aorta endothelium cells in vitro [38]. Furthermore, CEdG was detected in human kidneys and aorta by immunohistochemistry with the monoclonal CEdG antibody [39]. In this study, increased nuclear DNA glycation was observed in the kidney cells from patients with diabetic nephropathy and in the aorta of diabetic and non-diabetic hemodialysis patients, compared to control. Thus, CEdG is the only DNA-AGE, which has been detected in vivo so far.

The relevance of the different carbonyl compounds as precursors for DNA glycation is not clear. Cellular concentrations were determined for some carbonyl precursors: 19.6 pmol methylglyoxal/10^6 cells were measured in endothelial cells in vitro, whereas glyoxal and 3-deoxyglucosone were below the detection limit of 11 and 21 pmol/10^6 cells, respectively. Intracellular MG concentration increased when the cells were cultured under high glucose concentration [40]. In another study, whole rat embryos were cultured in the presence of low and high glucose concentrations. Whereas methylglyoxal and glyoxal concentration in the embryo was unchanged under hyperglycemic conditions, 3-deoxyglucosone concentration increased significantly. High 3-deoxyglucosone concentrations were also associated with embryonic dysmorphogenisis [9]. Although glucose itself is readily phosphorylated after cellular uptake, intracellular glucose concentrations in bovine endothelial cells reflected the glucose concentrations in the medium, and levels between 5.1 and 55.2 pmol/μg were measured [41]. Furthermore, elevated concentrations of fructose, fructose diphosphate and triose phosphate were observed under hyperglycemic conditions in vitro [42]. Thus, it was hypothesized, that fructose, phosphorylated sugars, and sugar degradation products, as well as methylglyoxal, are important precursors for intracellular formation of protein AGEs [40,41]. However, information on the nuclear concentration of sugars and sugar degradation products is rare. In the nucleus, DNA itself must be considered as an important carbonyl precursor. Oxidation reactions release sugar degradation products from the DNA backbone, which then, in reverse, may directly glycate the DNA [33].

In vitro, CEdG is formed from various precursors. Therefore, this DNA-AGE seems to be a suitable marker to monitor DNA glycation in general, independent from the glycation precursor, and a LC–MS/MS method was developed to measure CEdG formation in vitro and in vivo [24].

5. LC–MS analysis of DNA-adducts

For a long time, mass spectrometry has been a standard method for the identification of novel nucleobase adducts formed by exogenous or endogenous genotoxic compounds. After the development of LC–MS coupling techniques and improvement of their sensitivity and performance, LC–MS has now become a leading method for the qualitative and quantitative analysis of DNA-adducts in vitro or in vivo [43,44]. The analysis of DNA modifications by LC–MS was recently reviewed in detail [44]. In contrast to GC–MS, which usually requires derivatization of the DNA-adducts prior to analysis, the modified nucleobases can be analyzed by HPLC without derivatization. Thus, LC–MS analysis is less time consuming and less prone to artifacts, deriving from the derivatization procedure [45]. On the other hand, gas chromatography provides better peak resolution and, in many cases, higher sensitivity than HPLC. There are numerous LC–MS methods, which were developed for the analysis of DNA modifications, for example, for adducts derived from oxidation (8-hydroxy-2′-deoxyguanosine), methylation, heterocyclic aromatic amines, or PAH [43,44,46–49]. Prior to HPLC analysis, the respective adducts are generally released from the DNA back bone. Strong acid (e.g. 60% formic acid for 30 min at 140 °C) hydrolyzes DNA completely

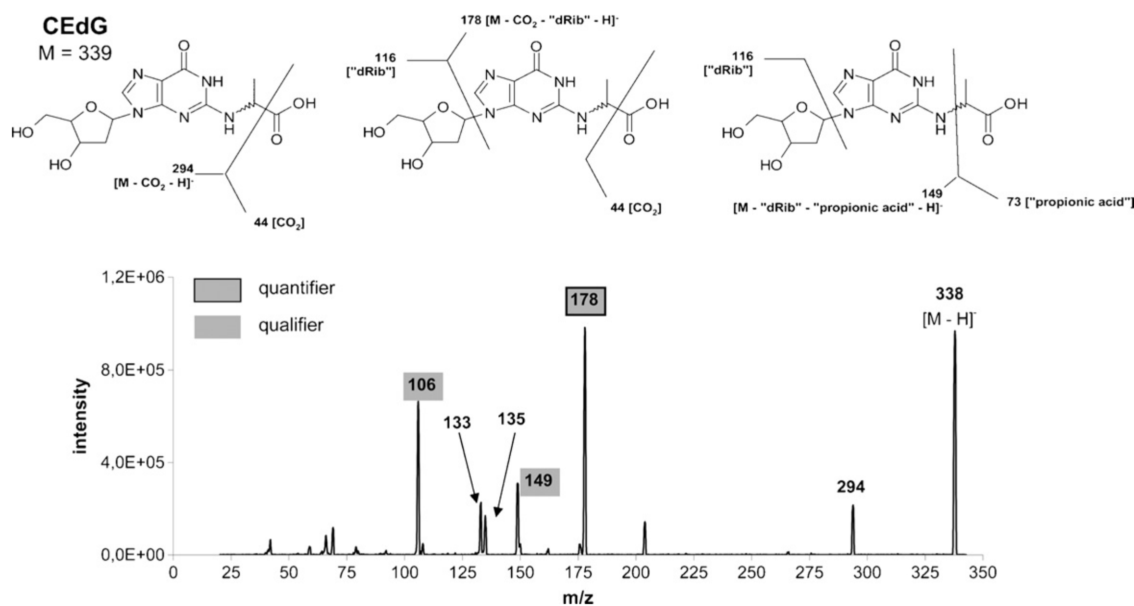

Fig. 1. Product ion scan of CEdG showing the molecule ion [M–H]$^-$ with 338 *m/z* and six typical fragment ions. The three most intense fragment ions are used to identify the analyte (qualifier). Quantification is achieved via the most intense fragment ion (quantifier). During fragmentation the molecule looses carbon dioxide and the deoxyribose.

to the nucleobases [50]. In some cases, particularly when guanine or adenine are modified in position N-3 or N-7, the nucleobase adduct can be selectively released from the DNA by thermal hydrolysis, whereas mild acid hydrolysis selectively cleaves the purine bases from the DNA backbone [51]. Furthermore, DNA repair enzymes, mainly formamidopyrimidine glycosylase (Fpg), can be used to cleave selectively nucleobase adducts from the DNA [50]. Alternatively, modified 2′-deoxynucleosides can be analyzed, which are commonly released from the DNA by enzymatic hydrolysis. For complete hydrolysis, a combination of different enzymes is required, such as nuclease S1, which cleaves the phosphodiester bonds releasing 2′-deoxynucleotides, combined with snake venom phosphodiesterase and alkaline phosphatase, which liberate the 2′-deoxynucleosides [52]. An up to 10^8-fold excess of unmodified nucleobases as well as salts originating from sample workup and other contaminants can largely decrease sensitivity in mass analysis. An enrichment of the modified nucleosides prior to LC–MS analysis can be achieved by solid phase extraction and/or immunoaffinity chromatography [38,53,54]. These clean-up techniques were further improved by on-line coupling, using two-dimensional chromatography with automatic column-switching [43,55]. Direct analysis of the nucleoside adducts from the digested DNA without further purification has also been described [56]. For chromatographic separation of the modified nucleosides and nucleobases, usually reversed phase columns with RP-18 or RP-8 stationary phases are applied [24,27,53,57]. The adducts are commonly eluted with aqueous phase—acetonitrile gradients [24,27,57,58]. In some cases, methanol is used instead of acetonitrile [54], but the formation of artifact adducts from methanol has been described [59]. After liquid chromatography, DNA adducts are often ionized by electrospray ionization (ESI) [60]. ESI-MS compatible aqueous phases are, for example, ammonium acetate buffer, ammonium formate buffer, diluted formic acid or acetic acid. The advantage of ESI is that it can be directly coupled to LC, and that it leads to negligible fragmentation of the analytes. Depending on the LC–MS instrument and the composition of the solvents, either positive or negative ionization lead to higher signal intensity. Because of the labile glycosidic bond and relatively high polarity of modified DNA bases, atmospheric pressure chemical ionization (APCI) is only exceptionally used for their analysis [61].

Due to rather complex matrix composition, tandem MS (MS/MS) is required for the unambiguous identification of the DNA adducts. For this purpose, most commonly triple quadrupole mass spectrometers are applied, which are used in the multiple reaction monitoring (MRM) mode. In this set-up, the first quadrupole serves as a mass filter, which allows only the passage of the unfragmented parent ion [M + H]$^+$ (positive mode) or [M–H]$^-$ (negative mode). The second quadrupole is filled with inert gas and used as a collision cell, where collision induced dissociation (CID) takes place. Depending on the collision energy, the parent ion is fragmented into several daughter ions, which enter the third quadrupole. In the third quadrupole, typically the three most abundant and specific mass transitions are selected as qualifiers. The presence and the ratio of the qualifiers allow unambiguous identification of the analyte in the sample. The identification of CEdG by MS/MS in the MRM mode is shown in Fig. 1. Other MS/MS combinations are, for example, quadrupole ion-trap mass analyzer [62] or combined quadrupole/time-of-flight analyzer (Q-TOF) [63].

For quantification in the MRM mode, the most abundant daughter ion is selected as quantifier. Since quenching in the ion source by co-eluting matrix components is often observed,

Table 1
AGEs of nucleobases and their derivatives which have been identified after isolation from model reaction mixtures by preparative HPLC

Carbonyl component	Nucleobase derivative	Product[a]	Reference	Comment
Glucose	Guanosine	N^2-(1-Carboxy-3,4,5-trihydroxypentyl)-guanosine ($CTPG_{A,B}$)	[15]	
Glucose	Guanosine	2-(β-D-Glucopyranosylamino)-guanosine	[13]	Only at elevated temperatures
Glucose	Guanosine	N^2-(1-Carboxymethyl)-guanosine (CMG)	[20]	In the presence of oxygen; also from glyoxal at elevated temperatures
Glucose	2′-Deoxyguanosine	2-(β-D-Glucopyranosylamino)-guanine	[13]	Only at elevated temperatures
Glucose	2′-Deoxyguanosine	N^2-(1-Carboxy-3,4,5-trihydroxypentyl)-2′-deoxyguanosine ($CTPdG_{A,B}$)	[14]	In the presence of propylamine
Glucose	9-Methylguanine	N^2-(1-Carboxymethyl)-9-methylguanine	[21]	
Glyceraldehyde	2′-Deoxyguanosine	N^2-(1-Carboxyethyl)-2′-deoxyguanosine ($CEdG_{A,B}$)	[17]	
Glyceraldehyde, dihydroxyacetone	2′-Deoxyguanosine	N^6-[2-(N^2-2′-Deoxyguanosyl)-propionyl]-lysine	[66]	In the presence of lysine; potential DNA-protein cross-link product
Glyoxal	2′-Deoxyguanosine, DNA	Cyclic adduct of glyoxal with 2′-deoxyguanosine (gdG)	[52]	
Glyoxal	2′-Deoxycytidine, DNA	2′-Deoxyuridine, 5-hydroxyacetyl-2′-deoxycytidine	[52]	
Glyoxal	2′-Deoxyguanosine, 2′-deoxyadenosine, 2′-deoxycytidine, cytidine and thymidine	Cyclic adduct of glyoxal with 2′-deoxyguanosine (gdG), N^6-(hydroxyacetyl)-2′-deoxyadenosine, 5-hydroxyacetyl-2′-deoxycytidine	[27]	
Cyclic adduct of glyoxal with 2′-deoxyguanosine	2′-Deoxyguanosine, 2′-deoxyadenosine, 2′-deoxycytidine	Imidazole[1,2-*a*]purine conjugate of dG, dA and dC	[67]	
Methylglyoxal	2′-Deoxyguanosine	N^2,7-Bis(1-hydroxy-2-oxopropyl)-2′-deoxyguanosine$_{A,B,C,D}$	[23]	Also formed from 1,3-dichloropropene epoxide
Methylglyoxal	2′-Deoxyguanosine, 2′-deoxyadenosine	$CEdG_{A,B}$, N^6-(1-Carboxyethyl)-2′-deoxyadenosine ($CEdA_{A,B}$)	[24]	
Methylglyoxal	Guanosine	Cyclic adduct of methylglyoxal with guanosine (mG)	[22]	
Methylglyoxal	2′-Deoxyguanosine, DNA	Cyclic adduct of methylglyoxal with dG (mdG)	[35]	
Phosphoglycol-Aldehyde	2′-Deoxyguanosine, DNA	Cyclic adduct of glyoxal with 2′-deoxyguanosine (gdG)	[65]	Phosphoglycolaldehyde is an oxidation product of 2′-deoxyribose in DNA
Ascorbic acid, dehydroascorbic acid or xylosone	Guanosine	N^2-(1-Carboxyethyl)-guanosine ($CEG_{A,B}$) N^2-(1-Carboxy-3-hydroxypropyl)-guanosine ($CHPG_{A,B}$)	[19]	
3-Deoxyglucosone	2′-Deoxyguanosine	N^2-(1-Oxo-2,4,5,6-hydroxyhexyl)-2′-deoxyguanosine$_{A,B}$	[68]	Only at elevated temperatures

The description of diastereomers is assigned by the suffices A–D.
[a] In some cases authors used different nomenclature according to IUPAC.

the use of a standard of the same structure, which is labeled with stable isotopes, is required. By adding the labeled standards prior to LC–MS/MS analysis, quenching effects, variations in the response of the mass spectrometer and losses during work-up can be efficiently compensated. The labeled standards contain most commonly several ^{15}N- or ^{13}C-isotopes resulting in a mass difference of 3–5 units compared to the analytes. For example, [$^{15}N_5$]8-oxo-2′-deoxyguanosine and [^{13}C,$^{15}N_2$]8-oxoguanine were applied for the quantification of oxidized nucleobases and deoxynucleosides in human urine [64]. The use of deuterated compounds can be limited by D/H exchange during sample work-up and, thus, loss of deuterated standard.

6. Analysis of DNA-AGEs by HPLC and LC–MS/MS

For many years, HPLC has been an essential tool for the isolation of novel DNA-AGEs. In model experiments, nucleobases, nucleotides, 2-deoxynucleotides, nucleosides, or derivatives thereof, have been incubated in the presence of carbonyl compounds, and the reaction has been monitored by HPLC. Since nucleobases display characteristic UV absorption spectra, new reaction products can be efficiently detected by UV or diode array detection; the separation is most commonly achieved on RP-material. New nucleobase-bound AGEs have been subsequently isolated by preparative HPLC under similar separation conditions as used for analytical monitoring. Large

scale preparation of new adducts has been carried out at elevated temperatures to increase the yield for spectroscopic structure elucidation. The AGE-adducts of nucleobase derivatives isolated by HPLC for structure elucidation are summarized in Table 1. Furthermore, HPLC/UV was used to investigate the formation of nucleobase-AGEs dependent on reaction parameters or reaction partners [12,16,52,65]. Thereby, a direct link between oxidative stress and AGE formation was found by the detection of DNA-AGEs after oxidative DNA damage [62].

In several studies, LC–MS/MS was used to detect and quantify DNA-AGEs in vitro and in vivo. Dennehy and Loeppky developed a LC–MS/MS method for quantification of gdG, the cyclic adduct of glyoxal with 2′-deoxyguanosine [59]. Because of the low stability of gdG, the DNA was enzymatically hydrolyzed under mildly acidic conditions. The deoxynucleosides were separated on a RP-18 column using an acetonitrile-water gradient as eluent containing 0.1% formic acid for improved ionization. Under these conditions, the stereoisomers of the gdG were not separated, but co-elution with the unmodified nucleosides could be avoided. The analytes were detected in the positive ion mode by a triple quadrupole mass spectrometer after electrospray ionization. The mass transition $326 \rightarrow 210$ *m/z*, which accounts for the loss of deoxyribose, was used in the SRM for peak identification. For quantification, an isotope labeled standard derived from 8-^{13}C-7,9-^{15}N-2′-deoxyguanosine was prepared, which co-eluted with the analyte. The method was then applied to quantify gdG in DNA which had been pre-incubated with glyoxal in vitro. Furthermore, about 4–11 gdG adducts/10^6 nucleotides were detected in liver DNA of rats which were administered *N*-nitrosodiethanolamine, a carcinogenic glyoxal precursor.

Olsen et al. developed a LC–MS/MS method, which covers, additionally to gdG, *N*6-(hydroxyacetyl)-2′-deoxyadenosine (gdA) [27]. The nucleosides were separated on a RP-18 column eluting with an acetonitrile–ammonium acetate buffer gradient. Mass detection was performed with tandem quadrupole after ESI in the positive mode and nucleosides were obtained from DNA by enzymatic hydrolysis at pH 6.5. Peak identification was achieved in the MRM using the transitions $326 \rightarrow 210$ (gdG) and $310 \rightarrow 194$ (gdA) *m/z*, respectively, both resulting from the loss of deoxyribose. The modification rate was determined by external standard. Thus, about 4000 adducts gdG/10^5 nucleotides and 0.2 adducts gdA/10^5 nucleotides were detected in DNA which had been incubated with glyoxal in vitro for several days.

Methylglyoxal adducts of nucleosides were first analyzed with LC–MS/MS by Schneider et al. [23]. Mass analysis was performed on a triple quadrupole after HPLC separation on a RP-18 column with a gradient of 0.1% trifluoroacetic acid and acetonitrile followed by ESI in positive and negative ion mode. The method was used to detect the methylglyoxal bis-adduct N^2,7-bis(1-hydroxy-2-oxopropyl)-2′-deoxyguanosine derived from the incubation of 2′-deoxyguanosine with methylglyoxal or 1,3-dichlorpropene epoxide.

Frischmann et al. used LC–MS/MS for the analysis of methylglyoxal adducts of 2′-deoxyguanosine or DNA using a triple quadrupole MS after ESI. Negative ionization increased sensitivity compared to the positive ion mode. A Q1 fullscan of enzymatically hydrolyzed DNA, which had been incubated in the presence of 100 mM methylglyoxal, showed four major peaks, corresponding to the mono- and bis-adducts from methylglyoxal and 2′-deoxyguanosine, respectively. The mono-adducts were identified as $CEdG_{A,B}$ and N^6-(1-carboxyethyl)-2′-deoxyadenosine ($CEdA_{A,B}$), respectively. For the bis-adducts, structures were postulated, in which a second molecule methylglyoxal is reversibly bound to N7 of $CEdG_{A,B}$ or $CEdA_{A,B}$. The products were separated on a RP-8 column with an ammonium formate buffer—acetonitrile gradient as eluent, and the peaks were identified in the MRM using the three most intensive and characteristic mass transitions as qualifiers. This method allowed the detection of all four adducts in DNA, which had been incubated with 10 mM methylglyoxal, whereas only $CEdG_{A,B}$ were identified, when DNA had been reacted with physiological concentrations of methylglyoxal (10 μM, Fig. 2) [24].

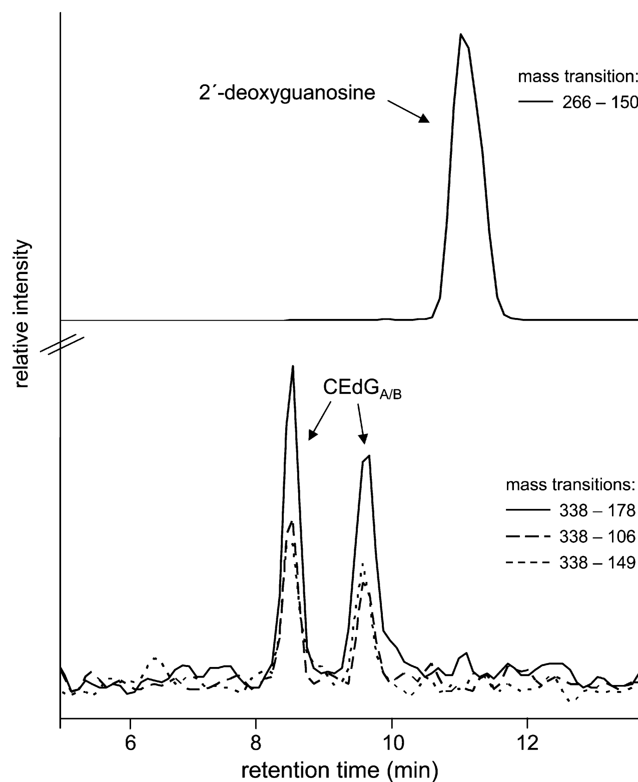

Fig. 2. DNA was reacted with 10 μM methylglyoxal for one week at 37 °C. After incubation, the DNA was enzymatically hydrolyzed resulting in a mixture of free nucleosides. The investigation of the mixture via LC–MS/MS revealed the formation of $CEdG_{A,B}$ (lower chromatogram, mass transitions 338-178, 338-106, 338-149). The upper chromatogram shows the excess of unmodified 2′-deoxyguanosine (mass transition 266-150).

$CEdG_{A,B}$, which may derive from various carbonyl precursors, such as methylglyoxal, glucose, or glyceraldehyde, were also detected in the genomic DNA of cultured human aortic smooth muscle cells [38]. Prior to LC–MS/MS analysis, the DNA was enzymatically hydrolyzed and enriched by immunoaffinity chromatography, using an antibody specific for

$CEdG_{A,B}$. The nucleosides were separated on a RP-18 column, with an ammonium acetate–acetonitrile gradient. Mass analysis was performed on a triple quadrupole after negative ESI in the MRM mode (three qualifiers).

7. Conclusion

Due to new developments in chromatography, LC–MS coupling, ionization and mass analysis, LC–MS/MS has become a very sensitive and reliable method for the analysis of DNA adducts. The use of isotope labeled standards also allows a highly accurate quantification. LC–MS/MS has been successfully applied for the analysis of DNA-AGEs and nucleobase-AGEs which are formed in vitro. It can be expected that LC–MS/MS will also be a valuable tool to detect and quantify DNA-AGEs in vivo. These data will promote our knowledge on the contribution of DNA glycation in aging and disease.

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