Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 797 (2003) 321–329
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Separation procedures capable of revealing DNA adducts

Yukihiro Esaka*, Shinsuke Inagaki, Masashi Goto

Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan

Abstract

Detection and quantification of DNA adducts are very important in relation to diseases such as cancer. Both high sensitivity and high selectivity are required for the detection of DNA adducts because the content of adducts in DNA is very small compared with those of normal bases and only small amounts of DNA samples are available for analysis in general cases. In this paper are described separation procedures such as liquid chromatography, gas chromatography and capillary electrophoresis combined with a detection and identification method such as ^{32}P-postlabeling, mass spectrometry, electrochemical detection, fluorescence detection and immunoassay. The merits and demerits of the procedures are also discussed.

Keywords: Review; DNA adducts

Contents

Abbreviations: AAF, *N*-acetyoxy-*N*-acetyl-2-aminofluorene; APCI, atmosphere pressure chemical ionization; ATP, 2′-adenosine triphosphate; BA, bromoacrolein; BODIPY® FL EDA, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionyl ethylenediamine hydrochloride; BPDE, benzo[*a*]pyrene diol epoxide; β-CD, β-cyclodextrin; CE, capillary electrophoresis; CEC, capillary electrochromatography; CF-FAB, continuous flow fast atom bombardment; CZE, capillary zone electrophoresis; dAMP, 2′-deoxyadenosine 5′-monophosphate; dCMP, 2′-deoxycytidine 5′-monophosphate; dG, 2′-deoxyguanosine; dGMP, 2′-deoxyguanosine 5′-monophosphate; dT, 2′-deoxythymidine; DNA, deoxyribonucleic acid; ECD, electrochemical detection; EI, electron impact; ESI, electrospray ionization; FAB, fast atom bombardment; FITC, fluoresceine isothiocyanate; HPLC, high-performance liquid chromatography; IS, internal standard; LIF, laser-induced fluorescence; MDA, malonedialdehyde; MEKC, micellar electrokinetic chromatography; MS, mass spectrometry; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; PEG, polyethylene glycol; PEI, polyethylene imine; PGE, phenylglycidyl ether; PhIP, phenyl imidazo[4,5-b]pyridine; SIM, selected ion monitoring; Tg, thymine glycol; TLC, thin layer chromatography; TSI, thermospray ionization; UV, ultra-violet

* Corresponding author. Tel.: +81-58-237-3931; fax: +81-58-237-5979.
E-mail address: esaka@gifu-pu.ac.jp (Y. Esaka).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S1570-0232(03)00607-X

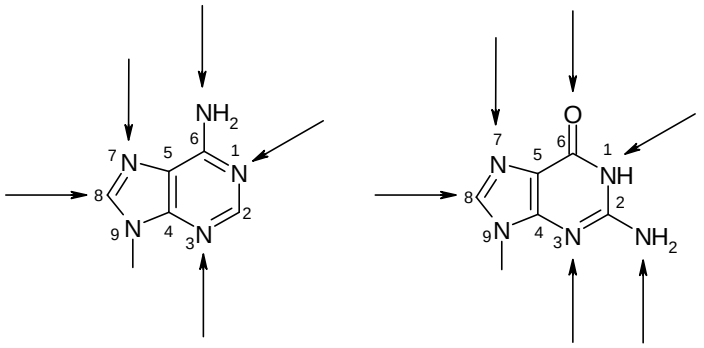
1. Introduction

Statistical studies for humans and investigations using laboratory animals reveal relationships between the occurrence of deoxyribonucleic acid (DNA) damage and exposure to certain chemicals, electromagnetic wave possessing a short wavelength and also stressful living. When DNA adducts, including decomposed forms of bases, are detected with remarkable frequency in our own DNA, we should recognize the following situations have arisen: (i) there are mutagenic chemicals, sources of dangerous electromagnetic wave or serious stresses to cause DNA mutation around our lives, (ii) the possibility of falling victim to serious diseases such as cancers caused by changes in the DNA sequence is increased drastically. We are required to decrease the quantity of mutagenic subjects in our surroundings and, if possible, to remove them completely. On the other hand, we should investigate the mechanisms expressing toxicity for each DNA adduct and also reveal the strength of the toxicity. Especially, transversion activity of each adduct must be considered from the viewpoint of carcinogenicity.

Fig. 1 shows damageable moieties of DNA bases. Generally, nitrogen and oxygen atoms on the bases are active sites and we have known addition reactions on N^2, N-3, O^6 and N-1 sites of guanine, N-1, N-3, N^6 and N-7 sites of adenine, O^2, N-3 and N^4 sites of cytosine and O^2 and O^4 sites of thymine to form several adducts. There are also some addition reactions on phosphate and deoxyribose moieties. Guanine is the most reactive base of the four DNA bases, and N^2-alkylated, O^6-alkylated guanines and C-8 hydroxylated guanine [8-hydroxyguanine (8-OH-Gua)] are well-known adducts [1,2]. 8-OH-Gua is considered to cause frequent G–T transversion. Additionally, active oxygens react with both guanine and 8-OH-Gua to yield 2-aminoimidazolone (Iz), which can cause complete G–C transversion [3,4]. On the other hand, exposure to some aldehydes and ultraviolet rays is responsible for crosslinking formation between the amino groups of bases in DNA and intrastrand crosslinking can cause miscopy of DNA [5]. In general, damage to DNA can increase frequency of miscopy of DNA. Many repair enzymes against damage to DNA work in our bodies, but sometimes repairs are impossible and then, apoptosis or flame shift mutations will occur. Almost all cancers start with changes in DNA sequences.

Generally, the amounts of such damaged bases in DNA of living organisms are very small and are reported to be some adducts per 10^6–10^8 normal bases. Additionally, the quantity of DNA sample obtained from tissues and bloods is some hundreds μg practically. Therefore, both highly sensitive and highly selective methods are required for analysis of adducts in DNA.

In this review are described separation procedures such as thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC) and



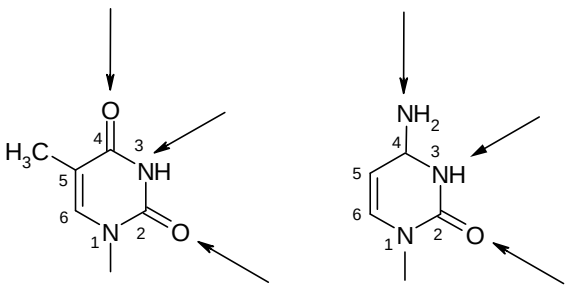


Fig. 1. Potential sites of addition reactions for the DNA bases.

capillary electrophoresis (CE) combined with several detection and identification methods in consideration of practical applications.

2. Chromatographic methods

DNA adducts are detected as the corresponding bases, nucleosides and nucleotides. TLC separation has been often used with radioisotopic labeling methods because TLC plates are suitable for photographic detection, which are popular for radioactive materials. GC is one of the most preferable separation methods for mass spectrometry (MS) detections, and GC-MS methods have been employed in analysis of DNA damages, although modification of targets is needed to make the targets volatile. HPLC will be highly advantageous for separation of compounds related to nucleic acids and thus, there are many applications using HPLC with several detection methods. The reversed-phase (RP) mode has been used in most cases. Nucleotides are often too hydrophilic to be separated with ODS columns as the most popular ones in RP-HPLC and thus, DNA adducts have been detected as nucleosides or bases in general.

2.1. ^{32}P-postlabeling method

The ^{32}P-postlabeling technique can be one of the most sensitive and widely accepted methods for the detection of very low levels of DNA adducts and has made a major contribution to this field [6,7]. In the original method introduced by Randerath et al., a DNA sample was digested with micrococcal endonuclease and spleen exonuclease, yielding deoxyribonucleoside 3′-monophosphates. The products are then labeled with ^{32}P at their 5′ end, using T_4 polynucleotide kinase and (γ-^{32}P) adenosine-5′ triphosphate (ATP), and separated by two-dimensional polyethyleneimine-TLC. DNA adducts were detected by auto-radiography. This method allows detection of one adduct per 10^5 normal nucleotides [6].

Marked improvement of sensitivity was achieved in the following modified method. One important refinement is the inclusion of a nuclease P1 digest before labeling [8]. The nuclease dephosphorylates normal deoxyribonucleoside 3′-monophosphates but seldom digests modified nucleotides. Deoxyribonucleosides without the phosphate moiety do not become substrates for phosphorylation by T_4 polynucleotide kinase. Consequently, only adducts which were not dephosphorylated in the previous step will be labeled as diphosphate forms possessing radioactivity. By removing background interference of a large amount of normal nucleotide, remarkable highly sensitive detection was achieved. This modified method allows detection of as little as one adduct per 10^7–10^{10} normal nucleotides [8]. Coupling of the ^{32}P-postlabeling technique with HPLC separation of DNA adducts results in a highly specific detection method (Fig. 2) [9–13].

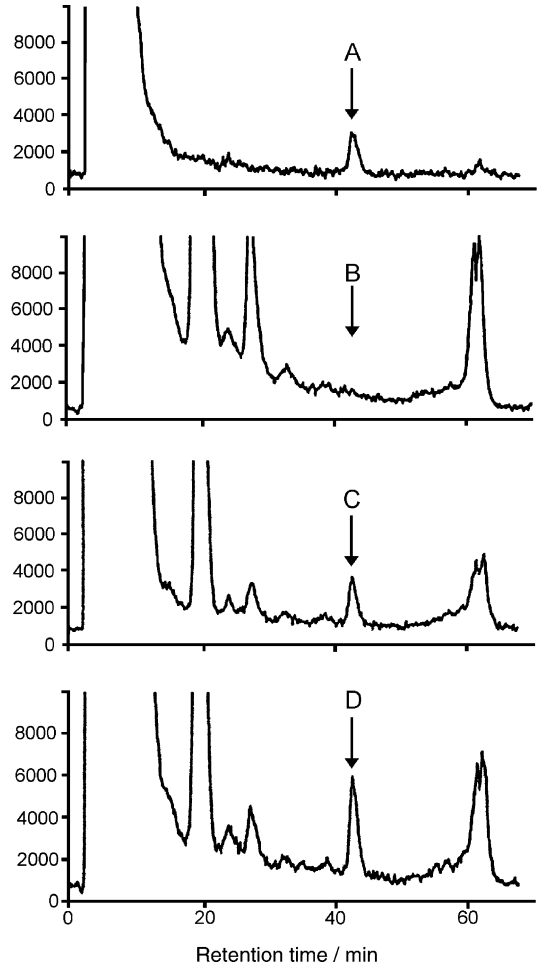


Fig. 2. Reversed-phase HPLC profiles of ^{32}P-postlabeled DNA adducts of acetoaldehyde in granulocytes DNA samples. (A) N^2-ethyl-2′-deoxyguanosine-3′-monophosphate standard; (B) DNA sample from a control subject; (C) DNA sample from an alcoholic patient; (D) sample in (C) + the adduct standard [13].

2.2. Mass spectrometry

The sensitivity of the ^{32}P-postlabeling method is excellent, but this method lacks the ability to provide structural information of the adducts. When the structures of DNA adducts are unknown or their standards were not obtained, MS is a powerful tool for the identification of DNA adducts [14–16]. MS also has offered good sensitivity toward DNA adducts. On the other hand, it should be mentioned that MS itself is a separation process as obviously suggested by the role of the first MS in MS/MS systems.

Lijinskey et al. used electron ionization (EI)-MS with isotope labeling to elucidate the reaction mechanism of nitrosodimethylamine to induce 7-methylguanine [17]. Induced 7-methylguanine was isolated from hydrolysates of treated rat liver DNA by column chromatography prior to EI-MS analysis. DNA adducts can be detected in urine also because of DNA repair system in cells where some modified bases are excised from DNA followed by their

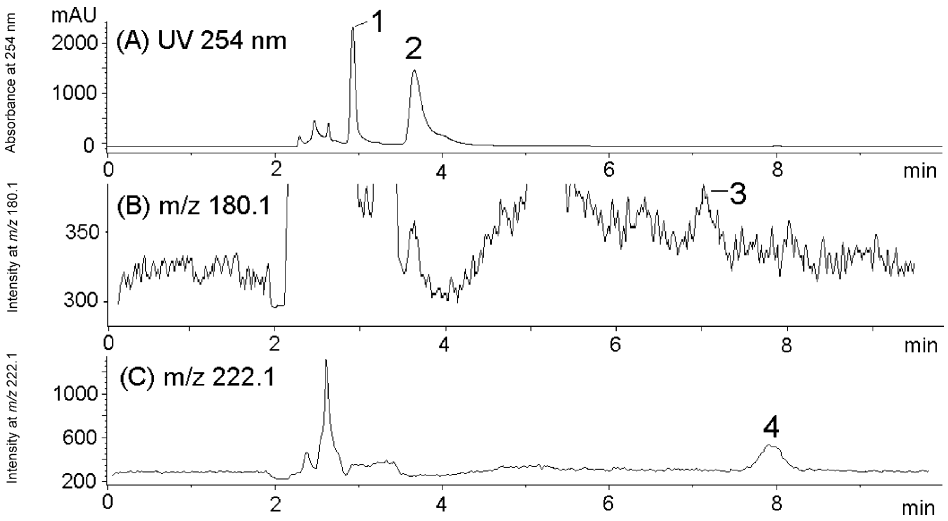


Fig. 3. Reversed-phase HPLC profiles of acid hydrolyzed samples of DNA isolated from HL-60 cells exposed to 50 mM acetaldehyde for 24 h. (A) UV detection, (B, C) MS detection. Peak identification: 1, guanine; 2, adenine; 3, N^2-ethylguanine; 4, 1,N^2-propanoguanine [32].

excretion in urine. 7-Methylguanine, N^2-methylguanine, N^2,N^2-dimethylguanine, 7-(2-hydroxyethyl)-guanine and N^2-ethylguanine were extracted from urine and detected using GC-EI-MS or GC-EI-MS/MS after chemical derivatization by heptafluorobutylic anhydride [18] or other reagents. Benzo[*a*]pyrene diol epoxide-guanine adducts and thymine glycol were also detected using GC-EI-MS [19]. The efficiency of the extraction procedure was increased by using immunoaffinity columns to purify the alkyl adducts [20,21].

First atom bombardment (FAB)-MS and FAB-MS/MS were used to detect and characterize DNA adducts. Adducts derived from reactions of a nucleoside and nucleotides with cyanoethylene oxide have been characterized with the help of FAB-MS and FAB-MS/MS [22,23]. Four amino polyaromatic hydrocarbon-DNA adducts were also identified and characterized by FAB-MS and FAB-MS/MS [24]. Continuous flow (CF)-FAB enabled low-level analyses required for this type of analysis [25]. HPLC-CF-FAB-MS was employed to examine the products derived from the reaction of *N*-acetoxy-*N*-acetyl-2-aminofluorescein (AAF) with calf thymus DNA [26].

HPLC-thermospreay ionization (TSI)-MS has been used to identify 2-bromoacrolein [27] and phenyl glycidyl ether (PGE) adducts of nucleosides [28]. HPLC-TSI-MS was also used in the analysis of malondialdehyde-guanine adducts extracted from human urine [29]. TSI-MS/MS was used to confirm the structure of the adducts.

HPLC-ESI-MS has been used to detect modified guanines following acid hydrolysis of DNA. Compared with the nucleoside form, DNA bases have much higher ionization efficiency; therefore, highly sensitive detection has been achieved [30]. Using the ESI-MS method, O^6-methylguanine and N^7-methylguanine were detected in calf thymus DNA treated by *N*-nitrosodiethylamine with UV irradiation [31]. Most recently, N^2-ethylguanine and cyclic 1,N^2-propanoguanine adducts were detected in cultured HL-60 cells which were treated by acetaldehyde (Fig. 3) [32]. The limit of detection has been 1.0×10^{-10} M (2 fmol) and 7.5×10^{-10} M (15 fmol), respectively, using the selective ion monitoring (SIM) mode (these values are improved data in our additional study about [32]). 8-Hydroxydeoxyguanosine (8-OH-dG) in human urine was also detected by HPLC-ESI-MS supported by solid-phase extraction [33].

2.3. HPLC-fluorescence detection

O^6-Methylguanine and 7-methylguanine were detected based on their own fluorescence, following HPLC separation [31,34]. The limit of detection of O^6-methylguanine was better than 100 fmol and this method allows detection of one O^6-methylguanine per 10^5 normal guanine [31].

Nucleotides can be labeled with dansyl chloride or fluorescein isothiocyanate following treatment with ethylenediamine as a linker between the chromophores and the phosphate moiety of nucleotide [35–39]. This method allows detection of one O^6-methyl-5′-deoxyguanosine-2′-monophosphate (dGMP) per 10^8 normal nucleotides [40]. The limit of detection by this method is comparable to that by the ^{32}P-postlabeling method.

2.4. HPLC-electrochemical detection

Electrochemical detection (ECD) is a sensitive and selective method for the detection of electrochemically active compounds. Guanine has the lowest oxidation potential and thus, is the most damageable base among the four DNA bases under oxidaizable conditions. Many kinds of adducts related to guanine have been found in DNA and often have electrochemical activity. Therefore, HPLC-ECD is

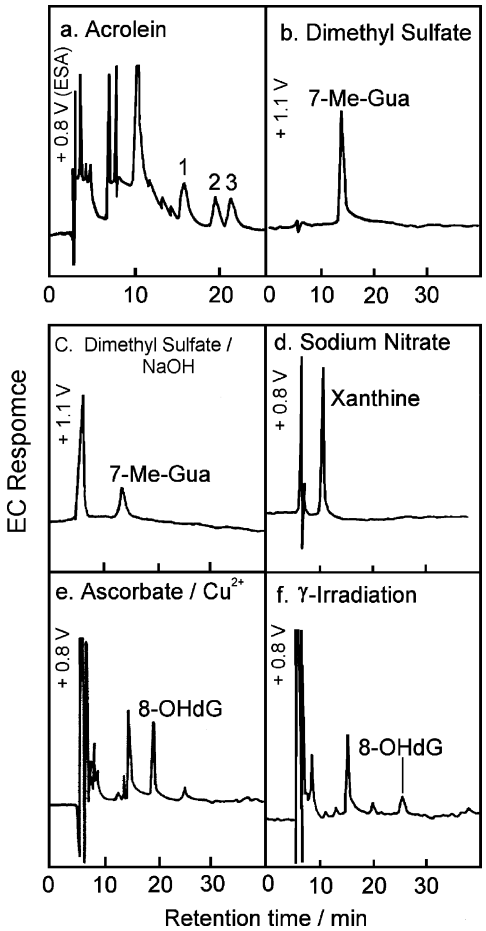


Fig. 4. HPLC-EC chromatogram of carf-thymus DNA treated with the indicated mutagens. Notations: m^7gua, 7-methylguanine; oh^{8}dG, 8-hydroxydeoxyguanine [41].

an appropriate method for the detection of guanine adducts [41].

8-OH-Gua is a major oxidative adduct formed by active oxygens-induced damage toward DNA and a highly electrochemical active compound [42]. The amount of the adduct in DNA has been measured using HPLC-ECD and is used as a biomarker of oxidative DNA damage (Fig. 4). Other damaged bases such as 7-methylguanine, O^6-methylguanine, xanthine and three acrolein adducts in DNA generated by several mutagens were also detected using HPLC-ECD (Fig. 4) [41].

3. Electromigration methods

CE also has been studied as a separation tool for analysis of DNA adducts [43]. CE is a most suitable method for separation of charged analytes, so adducts can be detected as nucleotides. We can separate nucleosides and bases also by employing the micellar electrokinetic chromatography (MEKC) mode or operating the pH condition to charge the base moieties. Similar detection methods to those for HPLC are available. On-line pre-concentration methods such as stacking also have been employed in some studies because of the relatively low sensitivity in concentration for CE systems [44]. In this review, we will predominantly focus on works in which DNA adducts were analyzed as small molecules such as mononucleotides, nucleosides and bases and thus, we will not mention about gel electrophoresis here.

3.1. CE-UV absorbance detection

The most conventional detection method in CE is that based on UV-Vis absorbance. The concentration limit of this detection is, unfortunately, often inadequate for detection of DNA adducts in real samples because of the small light-path-length for detection in the CE systems.

Norwood et al. demonstrated capillary zone electrophoresis (CZE) separation of normal nucleotides and benzo[*a*]pyrene diol epoxide (BPDE)-dGMP and applied it to analyze hydrolysate of DNA using UV absorbance detection [45]. Using stacking for on-line concentration, they achieved about 200-fold concentration compared with the normal injection by siphoning. CZE separation of deoxynucleotides including N^2-ethyl-dGMP related to alcohol abuse was studied using polyethylene glycol as a buffer additive [46]. 8-OH-dG in K562 human hematopoietic cells was detected by CZE-UV absorbance detection [47].

A method for the MEKC separation of 15 urinary normal and modified nucleosides from cancer patients was developed (Fig. 5) [48]. The levels of modified nucleosides in

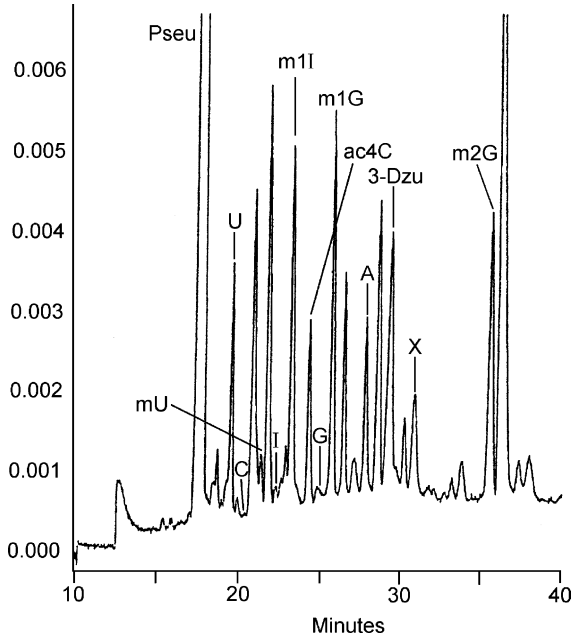


Fig. 5. Typical SDS-MEKC separation of normal and modified nucleosides extracted from urine of a cancer patient. Peak identification: Pseu, pseudouridine; Dhu, dihydrouridine; U, uridine; C, cytidine; mU, 3-methyluridine + 5-methyluridine; I, inosine; m1I, 1-methylguanosine; A, adenosine; 3-Dzu, 3-deazauridine; X, xanthosine; m2G, 2-methylguanosine; m6A, N^6-methyl-adenosine [48].

urine from cancer patients were higher compared with those in normal urine.

3.2. CE-laser induced fluorescence detection

Fluorescence derivatization of nucleotides for the analysis of DNA adducts using CE-laser induced fluorescence (LIF) detection has been developed by Giese et al. aiming to achieve comparable sensitivity with that of the ^{32}P-postlabeling method [35–39]. They performed fluorescence derivatization with dansyl chloride or fluorescein isothiocyanate in one step using a histidine-binding group [37] as well as a previous method including conjugation of ethylenediamine at the 5′-phosphate group of nucleotides followed by derivatization with the fluorescence reagents [35,36,38,39].

Very recently, Schmitz et al. reported a fluorescence derivatization method of nucleotide using 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionyl ethylenediamine, hydrochloride (BODIPY® FL EDA) and they detected etheno-2′-deoxyadenosine-5′-monophosphate (dAMP), 5-Me-2′-deoxycytidine-5′-monophosphate (dCMP), 8-OH-dGMP and various adducts of aristolonic acid generated in calf-thymus DNA by CE-LIF detection (Fig. 6) [49]. The chromophore of BODIPY® FL EDA possesses strong fluorescence contributing to high sensitivity of this method. This method allows detection of as little as 1.4 adducts per 10^7 normal nucleotides.

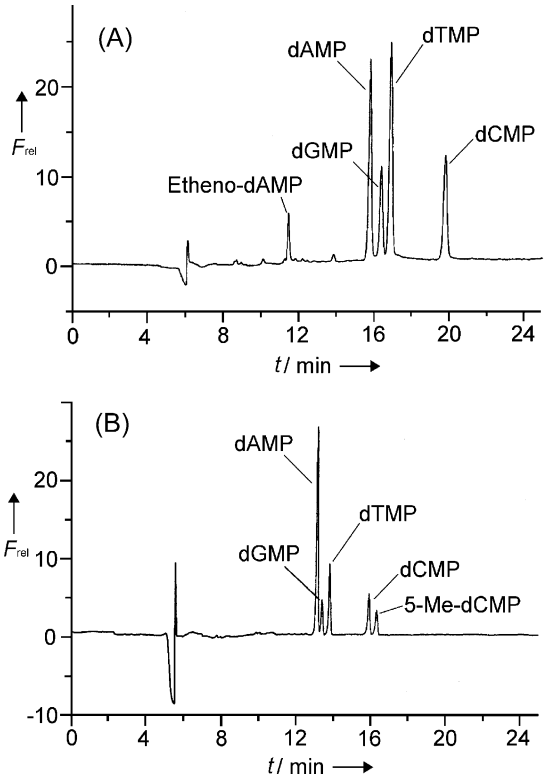


Fig. 6. LIF detection of etheno-dAMP (A) and 5-Me-dCMP (B) separated by SDS-MEKC [49].

Indirect fluorescence detection has been also studied to visualize chromatographic samples that cannot be detected without derivatization. Indirect detection of nucleotides, using a salicylate buffer as an electrophoretic buffer, has been reported [50].

3.3. CE-mass spectrometry

There is increasing expectation for CE-MS as a series of analysis systems of GC-MS and LC-MS. Interfaces of ESI and APCI for CE-MS are now on the market.

Vouros et al. analyzed AAF derived DNA adducts by CZE-CF-FAB-MS [51]. The use of whole capillary stacking has made it possible to improve the limit of detection in the nanomolar range. They also analyzed benzo[g]chrysene-DNA adduct using Capillary electrochromatography (CEC)-ESI-MS [52] and BPDE-dGMP using CZE-ESI-MS [53]. Phenylglycidyl ether (PGE)-DNA adducts were also analyzed using CZE-ESI-MS and CZE-ESI-MS/MS [54,55].

3.4. CE-electrochemical detection

ECD is an appropriate detection method for CE because, in principle, even under the conditions of a small volume of detection cells, there is little reduction in sensitivity of concentration. In addition, ECD can have higher selectivity with complex samples than optical techniques as a result of detection based upon the electrode potential.

Weiss and Lunte detected 8-OH-dG in human urine using CE-ECD [56]. This CE-ECD method has required solid-phase extraction only once, while LC methods have required complex and time-consuming double and triple column switching as well as multiple solid-phase extraction steps [42]. Detection of 8-OH-dG and N^2-ethyl-dG in DNA was studied using MEKC-ECD [57,58].

3.5. CE-radioisotope detection

On-line radioactivity detectors for CE were developed by Pentoney et al. [59,60] and its limit of detection of ^{32}P-labeled ATP was about 10^{-10} M. Recently, Schmitz et al. developed a detection method for ^{32}P-postlabeled DNA adducts by CE [61]. They separated ^{32}P-labeled nucleotides including some DNA adducts and this method allows detection of one adduct per 10^9 normal nucleotides.

4. Immunoassay

Immunoassay has been recognized as a powerful tool for the analysis of DNA adducts. Since Levine et al. prepared antibodies against far-UV-induced photoproducts of

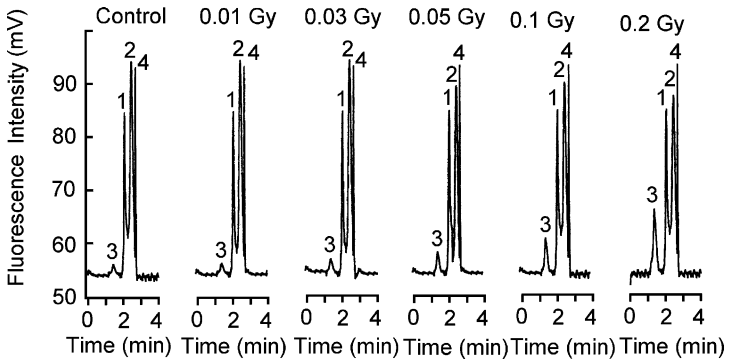


Fig. 7. Representative electropherograms showing the yield of Tg of in A519 human lung carcinome cells irradiated with increasing doses (0.01–0.2 Gy) from a ^{137}Cs γ-ray source. Peak identification: 1, fluorescently labeled secondary antibody; 2, the complex of primary and secondary antibody; 3, the complex of antigen with primary and secondary antibody; 4, free TMR [65].

DNA [62], several kinds of antibodies have been derived against oxidative and photo-induced DNA lesions, including thymine glycol (Tg), 7,8-dihydro-8-oxoadenine and cyclobutane-pyrimidine dimmers [63]. The main advantages of immunoassay are sensitivity, selectivity and simplicity once the corresponding antibodies have been generated. Imunoassay necessarily involves removal of free forms of labeled antibodies from the complex of the target antigens and the antibodies and, recently, chromatographic and electrophoretic methods have been employed for this purpose in some cases.

Radioimmunoassay combined with HPLC has been used to detect O^6-*n*-butyl-dG, O^2-*n*-butyl-deoxythymidine (dT), O^4-*n*-butyl-dT and O^6-methyl-dG [64,65]. Recently, Le et al. developed a highly sensitive assay for measuring DNA adducts that couples immunochemical recognition with CE-LIF (Fig. 7) [66]. This method requires only nanogram amounts of DNA and the limit of detection was one Tg per 10^9 bases. This method is not limited to detection of Tg but has been extended to other DNA adducts for which appropriate affinity probes are available [67,68].

5. Utility and quantification

When we choose an analysis method for adducts in DNA of cells, the first consideration will be the sensitivity of the method. The amount of adducts is very small compared with normal bases. A high degree of pre-concentration of adducts as trace components under co-existing of normal bases as main components will be practically difficult. Thus, we have to remove the main components before concentration, and such purification of trace components is troublesome in general. Additionally, only a small amount of real DNA samples is available, making it difficult to perform high enrichment of the samples mentioned above.

Because of the remarkable advantage in sensitivity based on high selectivity in labeling of target bases by enzyme reactions and the originally high sensitivity of detection with radioactivity, the ^{32}P-postlabeling method has been used in practical applications to DNA adducts, the structures of which were known [6–13]. Immunoassay also seems to have relatively sufficient sensitivity for analysis of real DNA samples
[62–68].

ECD methods have been often used to detect 8-OH-Gua considered as an important biomarker, because of its relatively high frequency in existence in DNA compared with other adducts and its high activity toward ECD to result in high sensitivity for the adduct [31,41,42,56–58].

When we only aim to detect known damaged bases, we can use excellent sensitivity with the SIM mode of HPLC-MS as mentioned above. HPLC-MS has a wide spectrum of analytes in damaged bases and derivatizations of targets are not required before analysis and thus, HPLC-MS will be used more and more for detection of damage in DNA [28–33].

Although chromatographic methods have been employed predominantly in analysis of DNA obtained from real samples, CE requires only minimum samples for analysis and this is a practically important advantage in performing measurements thoroughly for exact quantification since a small amount of DNA samples is available in many cases. For enhancement of the usefulness of CE in practical applications, however, we should employ some on-line enrichments and/or increase the sensitivity of detectors to make up for the lack of sensitivity in concentration. From this viewpoint, the LIF detection method seems one of the most promising detection methods for CE [49].

In the ^{32}P-postlabeling method, the ratio of the content of an adduct to that of the normal bases in DNA is obtained from comparison of radioactivity when labeling is done for only damaged bases with that when labeling is done for all bases. Calibration lines for quantification made by using standards compound of adducts have been used in almost analysis methods mentioned above. Some cases using internal standards (IS) are as follows. Matsuda et al. used deuterated N^2-ethyl-dG successfully as an IS for LC-MS measurement of N^2-ethyl-dG in urine to

perform a high degree of pre-concentration [69]. Schuker et al. employed 7-ethylguanine as IS for quantification of 7-methylamine by GC-MS [18]. Vaca et al. estimated the content of N^2-ethyl-dGMP in DNA of human white blood cells using the results of the ^{32}P-postlabeling method for both the original DNA sample and the N^2-ethyl-dGMP spiked DNA sample [12,13].

6. Evaluation of the analytical results

A result indicated as the ratio of content of an adduct to that of normal bases in DNA of an object is discussed from the viewpoint of comparison with the results of controls. Both the magnitude of frequency and the structure of the adduct show us how dangerous the present situation is, from the viewpoint of maintaining the life of the object. Some damaged bases have strong activities of transversion to cause miscopy of DNA sequences. Damaged bases will be biomarkers of the existence of mutagens in certain circumstances. Furthermore, if there is an obvious relationship between the frequency of occurrence of a certain disease such as cancer and the content of a certain adduct in DNA, the adduct can be a biomarker of the possibility of suffering from the “serious” disease in the near future. We can improve our lives using these biomarkers of the subjects.

Damaged bases in urea would be also similar markers as mentioned above, although we should recognize that the detected bases are not necessarily the original damaged forms existing in DNA and can be metabolites.

References

[1] R.A. Floyd, J.J. Watson, P.K. Wong, D.H. Altmiller, R.C. Richard, Free Rad. Res. Commun. 1 (1986) 163.
[2] M.A. Shigenaga, B.N. Ames, Free Rad. Biol. Med. 10 (1991) 211.
[3] K. Kino, I. Saito, J. Am. Chem. Soc. 120 (1998) 7373.
[4] K. Kino, I. Saito, J. Am. Chem. Soc. 121 (1999) 10836.
[5] T. Matuda, M. Kawanishi, T. Yagi, S. Matsui, H. Takebe, Nucl. Acids Res. 26 (1998) 1769.
[6] K. Randerach, M.V. Reddy, R.C. Guputa, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 6126.
[7] R.C. Gupta, M.V. Reddy, K. Randerath, Carcinogenesis 3 (1982) 1081.
[8] M.V. Reddy, K. Randerach, Carcinogenesis 7 (1986) 1543.
[9] F.-L. Chung, R. Young, S.S. Hecht, Carcinogenesis 7 (1989) 1291.
[10] R.G. Nath, H.-J.C. Chen, A. Nishikawa, R. Young-Sciame, F.-L. Chung, Carcinogenesis 15 (1994) 979.
[11] R.G. Nath, F.-L. Chung, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 7491.
[12] J.-L. Fang, C.E. Vaca, Carcinogenesis 16 (1995) 2177.
[13] J.-L. Fang, C.E. Vaca, Carcinogenesis 18 (1997) 627.
[14] P.B. Farmer, G.M.A. Sweetman, J. Mass Spectrom. 30 (1995) 1369.
[15] W.A. Apruzzese, P. Vouros, J. Chromatogr. A 794 (1998) 97.
[16] E.L. Esmans, D. Boroes, I. Hoes, F. Lemière, K. Vanhoutte, J. Chromatogr. A 794 (1998) 109.
[17] W. Lijinsky, J. Loo, A.E. Ross, Nature 218 (1968) 1174.
[18] D.E.G. Schuker, E. Bailey, S.M. Gorf, J. Lamb, P.B. Farmer, Anal. Biochem. 140 (1984) 270.
[19] A. Weston, M.L. Rowe, D.K. Manchester, P.B. Farmer, D.L. Mann, C.C. Harris, Carcinogenesis 10 (1989) 251.
[20] P.B. Farmer, D.E.G. Schuker, I. Bird, Carcinogenesis 7 (1986) 49.
[21] M. Bonfanti, C. Magagnotti, A. Galli, R. Bagnati, M. Moret, P. Gariboldi, R. Fanelli, L. Airoldi, Cancer Res. 50 (1990) 6870.
[22] J.M. Yates, S.C.J. Summer, M.J. Turner Jr., L. Recio, T.R. Fennell, Carcinogenesis 14 (1993) 1363.
[23] J.M. Yates, T.R. Fennell, M.J. Turner Jr., L. Recio, S.C.J. Summer, Carcinogenesis 15 (1994) 277.
[24] R.S. Anann, R.W. Giese, P. Vouros, Anal. Biochem. 191 (1990) 86.
[25] S.M. Wolf, R.S. Anan, P. Vourous, R.W. Giese, Biol. Mass Spectrom. 21 (1992) 647.
[26] S.M. Wolf, P. Vourous, Chem. Res. Toxicol. 7 (1994) 82.
[27] J.H.N. Meersman, T.R. Smith, P.G. Pearson, G.P. Meier, S.D. Nelson, Cancer Res. 49 (1989) 6174.
[28] F. Lemière, E.L. Esmans, W. Van Dongen, E. Van Den Eeckhout, H. Van Onckelen, J. Chromatogr. 647 (1993) 211.
[29] H.K. Jajoo, P.C. Burcham, Y. Goda, I.A. Blair, L.J. Marnett, Chem. Res. Toxicol. 5 (1992) 870.
[30] R. Singh, G.M.A. Sweetman, P.B. Farmer, D.E.G. Shuker, K.J. Rich, Chem. Res. Toxicol. 10 (1997) 70.
[31] S. Arimoto-Kobayashi, K. Kaji, G.M.A. Sweetman, H. Hayatsu, Carcinogenesis 18 (1997) 2429.
[32] S. Inagaki, Y. Esaka, Y. Deyashiki, M. Sako, M. Goto, J. Chromatogr. A 987 (2003) 341.
[33] H. Moriwaki, Anal. Sci. 16 (2000) 105.
[34] D.C. Herron, R.C. Shank, Cancer Res. 40 (1980) 3116.
[35] A.N. Al-Deen, D.C. Cecchini, S. Abdel-Backy, N.M.A. Moneam, R.W. Giese, J. Chromatogr. 512 (1990) 409.
[36] W. Li, A. Moussa, R.W. Geise, J. Chromatogr. 608 (1992) 171.
[37] P. Wang, R.W. Giese, Anal. Chem. 65 (1993) 3518.
[38] P. Wang, R.W. Giese, J. Chromatogr. A 809 (1998) 211.
[39] Z. Lan, X. Qian, R.W. Giese, J. Chromatogr. A 831 (1999) 325.
[40] R. Jain, M. Sharma, Canser Res. 53 (1993) 2771.
[41] J.-W. Park, K.C. Cundy, B.N. Ames, Carcinogenesis 10 (1989) 827.
[42] D. Germadnik, A. Pilger, H.W. Rüdiger, J. Chromatogr. B 689 (1997) 399.
[43] S.E. Geldart, P.R. Brown, J. Chromatogr. A 828 (1998) 317.
[44] R.L. Chein, D.S. Burgi, Anal. Chem. 64 (1992) 1046.
[45] C.B. Norwood, E. Jackim, S. Cheer, Anal. Biochem. 213 (1993) 194.
[46] Y. Esaka, S. Inagaki, M. Goto, M. Sako, Electrophoresis 22 (2001) 104.
[47] O. Ullrich, T. Grune, J. Chromatogr. B 697 (1997) 243.
[48] H.M. Liebich, G. Xu, C.D. Stefano, R. Lehmann, J. Chromatogr. A 793 (1998) 341.
[49] O.J. Schmitz, C.C.T. Wörth, D. Stach, M. Wießler, Angrew. Chem. Int. Ed. 41 (2002) 445.
[50] W.G. Kuhr, E.S. Yeung, Anal. Chem. 60 (1988) 2642.
[51] S.M. Wolf, P. Vourous, Anal. Chem. 67 (1995) 891.
[52] J. Ding, P. Vouros, Anal. Chem. 69 (1997) 379.
[53] J.P. Barry, C. Norwood, P. Vouros, Anal. Chem. 68 (1996) 1432.
[54] D.L.D. Deforce, F.P.K. Ryniers, E.G. Van den Eeckhout, Anal. Chem. 68 (1996) 3575.
[55] D.L.D. Deforce, F. Lemière, E.L. Esmans, A.D. Leenheer, E.G. Van den Eeckhout, Anal. Biochem. 258 (1998) 331.
[56] D.J. Weiss, C.E. Lunte, Electrophoresis 21 (2000) 2080.
[57] M. Goto, S. Inagaki, Y. Esaka, Anal. Sci. 17 (2001) 1383.
[58] S. Inagaki, Y. Esaka, M. Sako, M. Goto, Electrophoresis 22 (2001) 3408.
[59] S.L. Pentoney Jr., R. Zare, J.F. Quint, Anal. Chem. 61 (1989) 1642.
[60] S.L. Pentoney Jr., R. Zare, J.F. Quint, J. Chromatogr. 480 (1989) 259.
[61] O. Schmitz, E. Richter, Biomarkers 5 (2000) 314.

[62] L. Levine, E. Seaman, E. Hammerschlag, H. Van Vunakis, Science 153 (1966) 1666.
[63] J. Cadet, C. Anselmino, T. Douki, L. Voituriez, J. Photochem. Photobiol. B.: Biol. 15 (1992) 277.
[64] R. Saffhill, P.T. Strickland, J.M. Boyle, Carcinogenesis 3 (1982) 547.
[65] C.P. Wild, G. Smart, R. Saffhill, J.M. Boyle, Carcinogenesis 4 (1983) 1605.
[66] X.C. Le, J.Z. Xing, J. Lee, S.A. Leadon, M. Weinfeld, Science 280 (1998) 1066.
[67] H. Wang, J. Xing, W. Tan, M. Lam, T. Carnelly, M. Weinfield, X.C. Le, Anal. Chem. 74 (2002) 3714.
[68] H. Wang, M. Lu, M. Weinfield, X.C. Le, Anal. Chem. 75 (2003) 247.
[69] T. Matsuda, I. Terashima, Y. Matsumoto, H. Yabushita, S. Matsui, S. Shibutani, Biochemistry 38 (1999) 929.