Preparation of Cyclic-1,N2-propano-2'-deoxyguanosine-d7 as an Internal Standard for ESI-MS/MS Determination of DNA Damage from Acetaldehyde

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Abstract

Cyclic-1,N²-propano-2’-deoxyguanosine-d⁷ (CPr-dG-d⁷) was prepared as an isotopic internal standard (IS) for electrospray ionization tandem mass spectrometry (ESI-MS/MS) quantification of CPr-dG in DNA as a candidate cancer risk marker of acetaldehyde intake mainly from drinking. The deuterated compound was reasonably synthesized from acetaldehyde-d₄ and 2’-deoxyguanosine in deuterium oxide (D₂O), preventing the deuterium atoms of acetaldehyde-d₄ from being substituted by hydrogen atoms, which occurred seriously in aqueous synthesis media via keto–enol tautomerism. Furthermore, another deuterium atom was added from D₂O to form CPr-dG-d⁷. After four weeks of storage in H₂O at 10 °C, CPr-dG-d⁷ was found sufficiently stable for practical use. The calibration curve of CPr-dG by using a hydrophilic interaction chromatography–ESI-MS/MS system with CPr-dG-d⁷ as the IS showed sufficient linearity from 1.0 × 10⁻¹⁰ to 4.0 × 10⁻⁹ M with r² = 0.998.

**Keywords:** Cyclic-1,N²-propano-2’-deoxyguanosine-d⁷, Isotopic internal standard, ESI-MS/MS, Acetaldehyde-d₄, D₂O, Cancer risk marker
Introduction

**Acetaldehyde** is a naturally existing compound, mainly as a primary metabolite of alcohol. Recently, a positive relation between cancer-causing rate and alcohol intake has been confirmed, and **acetaldehyde** has been considered a significant mutagenic compound.\(^1\)–\(^9\) In particular, it was proven epidemiologically that humans possessing the hetero-type gene of type 2 acetaldehyde dehydrogenase (ALDH2), which are often found in Asians and indicate much weak ability to metabolize **acetaldehyde** to acetic acid, had much higher risks of developing cancer from drinking alcohol.\(^5\),\(^6\) Under such situation, the International Agency for Research on Cancer of WHO categorized **acetaldehyde** into group 2B, which means “a possible carcinogen,” and alcohol beverages into group 1, which means “carcinogenic to human.”\(^7\)–\(^9\) On the other hand, in our earlier work, cyclic-1,\(N^2\)-propano-2'-deoxyguanosine (CPr-dG) was generated predominantly as a stable adduct of **acetaldehyde** in the DNA of HL-50 cells cultured in the presence of **acetaldehyde**.\(^10\) Therefore, CPr-dG was shown as one of the final forms of **acetaldehyde** adducts in DNA, and it is a promising candidate for a cancer risk marker related to drinking. CPr-dG is formed from dG and two molecules of **acetaldehyde** via cyclization. **Acetaldehyde** is found in the smoke of cigarettes, and CPr-dG is formed from a molecule of crotonaldehyde (also found in the smoke).\(^11\),\(^12\) Therefore, CPr-dG could also be a cancer risk marker of smoking. Indeed, CPr-dG was detected in DNA in several human tissues.\(^13\),\(^14\)

In our previous work, a hydrophilic interaction chromatography (HILIC)–electrospray ionization tandem mass spectrometry (ESI-MS/MS) method was developed to determine the amounts of CPr-dG and \(N^2\)-ethyl-2'-deoxyguanosine (Et-dG); the latter is another stable adduct of **acetaldehyde** in DNA.\(^14\)\(^6\)\(^7\)\(^14\) Generally, quantitative analysis
using the ESI-MS/MS method requires an internal standard (IS), if possible, of isotopically labeled target compounds to correct any matrix interferences as well as relatively large intra- and inter-day variations of ESI-MS/MS signal intensity. In some previous works, CPr-dG-\(^{15}\!\!N_5\) and Et-dG-\(^{15}\!\!N_5\) prepared from 2’-deoxyguanosine-\(^{15}\!\!N_5\) (dG-\(^{15}\!\!N_5\)) were employed as the ISs in the LC-ESI-MS/MS measurements of CPr-dG and Et-dG.\(^{5,6,11}\) The costs of the preparation, however, became much higher, taking account of the price of dG-\(^{15}\!\!N_5\) as a starting material and yields in both synthesis and purification of the ISs. A sufficient amount of pure ISs is desired to perform a specific quantification of the adducts in DNA, especially for medical tests in the future. Therefore, deuterated CPr-dG as an IS was prepared using acetaldehyde-\(d_4\) and 2’-deoxyguanosine (dG) at a reasonable cost in this work. It should be emphasized that sufficient shifts of \(m/z\) between analytes and the corresponding ISs are required to prevent both MS signals of the analytes and the ISs from overlapping with the signals of their inherent isotopes. The stability of the deuterated ISs during storage as aqueous solutions and the linearity of the calibration curve of CPr-dG using those ISs were evaluated.

**Experimental**

**Reagents and chemicals**

Acetaldehyde, acetaldehyde-\(d_4\), deuterium oxide (D\(_2\)O), and adenosine-\(^{15}\!\!N_5\) were purchased from Kishida Chemical, CDN Isotopes, Euriso-Top, and Silantes, respectively. L-Arginine and dG were obtained from Tokyo Chemical Industry. Normal CPr-dG was synthesized according to a previous work\(^7\) and used as a standard preparation in this work. Acetonitrile of an LC/MS grade was purchased from Kanto
Chemical. Ammonium bicarbonate added to eluents as modifiers to increase ESI efficiency was obtained from Fluka. Other chemicals were of analytical grade. Sep-Pak® C18 (Vac 35 cc, 10 g) used in the purification of synthesized CPr-dG was available from Waters.

**Apparatus**

The deuteriation degree of synthesized samples was analyzed using a Shimadzu IT-TOF-MS detector with a Shimadzu Prominence HPLC system. In the analysis, separation [a reversed phase (RP)-mode] was performed using a TSKgel ODS-100V column (3 µm, 150 × 2.0 mm i.d., Tosoh Co.) and a mobile phase of MeOH:H₂O (milli-Q water) = 1:9 with a flow rate of 0.2 mL/min. The ESI conditions of the IT-TOF-MS system were as follows: nebulizer gas, 1.50 L/min; interface probe potential, 4.50 kV; needle potential, 0 kV (0 µA); CDL temperature, 200 °C; heat block temperature, 200 °C; and detector potential, 1.73 kV. On the other hand, quantitative LC-ESI-MS/MS measurements were performed using an AB SCIEX 3200 QTRAP detector with a Turbo ESI probe and a Shimadzu Prominence HPLC system. In the measurements, hydrophilic interaction chromatography (HILIC) was performed using a TSKgel Amide-80 column (3 µm, 150 × 2.0 mm i.d., Tosoh Co.) and a mobile phase of acetonitrile:H₂O (milli-Q) = 9:1, including 1.0 mM ammonium bicarbonate, with a flow rate of 0.2 mL/min. The condition of the 3200 QTRAP system was an optimized one in the previous work and set as follows: source mode, positive mode; curtain gas, 30 psi; ion spray voltage, 5500 V; turbo heater, 500 °C; nebulizer gas (GS1), 40 psi; heated gas (GS2), 40 psi; and collision gas (CAD), 5 psi. Moreover, selected reaction monitoring (SRM) mode was used for the detection of CPr-dG ([m/z]: 338.1 → 222.1) and CPr-dG-7 ([m/z]: 345.2 → 229.2).
Two diastereomers, (6S, 8S) form and (6R, 8R) form, of CPr-dG and those of the deuterated CPr-dG were fractioned from the synthesized compounds of CPr-dG and the deuterated CPr-dG, respectively, using a Shimadzu LC-10A HPLC system equipped with a Tosoh TSKgel ODS-100V column (5 µm, 150 × 4.6 mm i.d., Tosoh Co.) according to on-site chromatograms monitored at 254 nm. The reversed phase (RP-) HPLC was performed at 40 °C using a mobile phase of acetonitrile:H₂O (milli-Q) = 15:85 with a flow rate of 1.0 mL/min.

For all HPLC separations, samples were dissolved with acetonitrile:H₂O = 9:1 or 0:10 for HILIC or RP-HPLC, respectively, to avoid peak broadening in the initial stages of each chromatographic process.

Procedure

Synthesis of CPr-dG- d⁷: CPr-dG-d⁷ was synthesized according to Sako’s method¹⁵⁻¹⁸, but using deuterated reagents described as follows instead. Solid substances of 0.05 mmol dG and 0.1 mmol L-arginine were dissolved in 1.5 mL of D₂O in the presence of sodium dihydrogen phosphate and disodium hydrogen phosphate to be a 0.1 M phosphate buffer solution of pH 8.0. Then, 40 µL of acetaldehyde-d₄ was added to the solution, followed by incubation at 37 °C for 19 h. The product was purified using a Sep-Pak C18 cartridge in the same way as a regular CPr-dG preparation.

NMR analysis of the deuterated products: Deuterated positions in CPr-dG made from acetaldehyde-d₄ and dG were identified, as indicated in Fig. 1 based on the ¹H-NMR spectra of the products, in which proton signals in deuterated positions disappeared. Chemical shifts of ¹H positions were determined according to those of normal CPr-dG according to previous works,²,¹⁵,¹⁹⁻²¹ namely, [¹H NMR (400 MHz, (CD₃)₂SO) δ 1.21 (3H, dd, J = 6 Hz, NHCHMe), 1.41 and 2.01 (each 1H, br t and br d, J = each 12.5 Hz,
CHCH₂CHMe), 2.17 (1H, ddd, J = 3.5, 6.5, 13 Hz, 2′-CH₂), 2.50 (1H, overlapped with d-DMSO, 2′-CH₂), 3.51 (2H, m, 5′-CH₂), 3.70 (1H, m, NHCHMe), 3.79 (1H, dt, J = 2, 4.5 Hz, 4′-CH), 4.33 (1H, br s, 3′-CH), 4.92 (1H, br s, 5′-OH), 5.25 (1H, br s, 3′-OH), 6.09 (1H, dd, J = 1.5, 7.5 Hz, 2′-CH₂), 6.18 (1H, m, NCHOH), 6.63 (1H, d, J = 4.5 Hz, NCHOH), and 7.92 (1H, s, NCHN)].

Preparation of standard solutions of CPr-dG and CPr-dG-d₇: CPr-dG is formed via the addition of two molecules of acetaldehyde as two diastereomers, (6S, 8S) form and (6R, 8R) form, which are separated by RP-HPLC and eluted in this order.¹³ Each diastereomer of both normal CPr-dG and deuterated CPr-dG was fractionated from each of their synthetic substrates using RP-HPLC. A portion (0.1 mg) of the diastereomer mixture of normal CPr-dG or deuterated CPr-dG was injected to the HPLC system each time. Each standard solution of the diastereomers was prepared from the fractionated solutions. The concentrations of the diastereomers in the fractionated solutions were determined by HILIC based on a comparative estimate of peak areas between those of the fractionated solutions, areas of (6S, 8S) forms and (6R, 8R) forms, and that of CPr-dG diastereomers mixture of known concentration, assuming that all of the diastereomers have the same molar absorbance coefficient at 254 nm of the present detection wavelength. The HILIC system was employed to determine the trace amount of CPr-dG in DNA within our LS/MS/MS system⁷, and the diastereomers of CPr-dG were co-eluted with HILIC. The standard stock solutions of normal CPr-dG and deuterated CPr-dG were prepared at a concentration of 1.0 × 10⁻⁴ M.

In both cases of natural CPr-dG and deuterated one, the inter-lot variation in the formation ratios of the (6S, 8S) form and the (6R, 8R) form was not negligible on an empirical basis in our experiment. The peak area ratio of the present standard
preparation of CPR-dG was determined to be 1.75:1 [(6S, 8S) form:(6R, 8R) form] in the RP-HPLC separation. Therefore, a calibration curve of CPR-dG (diastereomer mixture) for the HILIC system was made using the deuterated CPR-dG of the diastereomer mixture prepared by mixing the (6S, 8S) form and the (6R, 8R) form in the ratio of 1.75:1, and the deuterated CPR-dG mixture was then used as the IS.

**Results and Discussion**

*Preparation of deuterated CPR-dG:* Fig. 1 shows a proposed reaction mechanism to generate CPR-dG-d7, whereby arginine (RND2) was used as a catalyst15-18, and the hydrogen atoms in R of RND2 would be omitted from the diastereomer products. In the first trial, distilled water was used as a reaction media as well as that in the preparation of normal CPR-dG. Six deuterium atoms were expected to be introduced to the resultant CPR-dG from two molecules of acetaldehyde-d4, yielding the deuterated products as suggested by Fig. 1 by replacing D2O with H2O. The deuterated products were analyzed by RP-HPLC–ESI-MS/MS. A chromatogram detected at 254 nm in the MS spectra of the two peak components was shown in Fig. 2. Both (6S, 8S) form (former peak) and (6R, 8R) form (latter peak) showed multiple peaks with a wide range of m/z values from 339.0 to 345.2 (as [M+H]+). This result strongly suggests that the replacement of deuterium atoms in acetaldehyde-d4 by hydrogen atoms of H2O occurred at a variety of deuteration degrees because of keto–enol tautomerism of acetaldehyde-d4 in H2O. As such, these deuterated products having a wide range of m/z as [M+H]+ species were not suitable as an IS to quantitate CPR-dG at all. Next, D2O was used to replace distilled water as a reaction solvent, in which the tautomerism was expected to avoid the replacement of deuterium atoms in acetaldehyde-d4. A juxtaposition of HPLC chromatogram and MS spectra of the products in D2O and those of normal CPR-dG were
shown in Fig. 3. In D$_2$O, CPr-dG-$d_7$ ($m/z = 345.2$ for [M+H]$^+$) was formed, assuring that not only the replacement of deuterium atoms in acetaldehyde-$d_4$ was avoided but also an additional introduction of the seventh deuterium atom at the 7-position in CPr-dG occurred. The position of the seventh one was determined by H-NMR by comparison of the H-NMR spectra of the deuterated CPr-dG with that of normal CPr-dG (see experimental section). Signals of $m/z = 338.1$ corresponding to the base signal of normal CPr-dG ($[\text{M}+\text{H}]^+$) were not observed in the MS spectra of the resultant deuterated CPr-dG diastereomers for both (6S, 8S) and (6R, 8R) forms. Likewise, signals of $m/z = 345.2$ were also not detected in the MS spectra of normal CPr-dG. Thus, the present deuterated product of CPr-dG consisting mainly of CPr-dG-$d_7$ would be a promising IS reagent in the quantitation of CPr-dG using ESI-MS/MS.

Generally, HILIC is a preferable alternative to connect to ESI-MS/MS compared with RP-HPLC in terms of ionization efficiency and sensitivity in detection. Indeed, HILIC–ESI-MS/MS measurements gave 26-times and 96-times larger peak areas for CPr-dG and Et-dG, respectively, in our previous work by changing the solvent ratios (acetonitrile:milli-Q water) of the mobile phase from 1:9 (RP-HPLC) to 9:1 (HILIC) using the same ESI-MS/MS system. Therefore, CPr-dG-$d_7$ was evaluated as an IS with an HILIC–ESI-MS/MS system. The two diastereomers of CPr-dG were co-eluted using the present HILIC systems and were determined as a mixture of both diastereomers. Thus, the difference in ESI efficiencies between the (6S, 8S) and (6R, 8R) forms was studied briefly. Each dilution of the stock solutions to $1.0 \times 10^{-9}$ M of normal CPr-dG diastereomers and those of the deuterated CPr-dG were carefully done. As a comparison, HILIC–ESI-MS/MS measurements of the four diluted solutions of each diastereomer under the previously optimized conditions were performed using Adenosine-$^{15}$N$_5$ with a concentration of $1.0 \times 10^{-9}$ M as the IS. The relative peak areas in the SRM
chromatograms were 0.69 ± 0.04 and 0.72 ± 0.03 for the (6S, 8S) form and (6R, 8R) form of normal CPr-dG, respectively, and 0.55 ± 0.01 and 0.56 ± 0.02 for the (6S, 8S) form and (6R, 8R) form of CPr-dG-d7, respectively, as average values ± RSD (n = 3). The ratios of relative peak areas for the (6S, 8S) and (6R, 8R) forms in the SRM chromatograms of normal CPr-dG (m/z = 338.1 → 222.1) and those of d7-CPr-dG (m/z = 345.2 → 229.2) became 0.96 and 0.93 based on the average values. As a matter of practice, there would be few differences in ESI efficiencies between the (6R, 8R) and (6S, 8S) forms for both normal CPr-dG and CPr-dG-d7. Therefore, with the present HILIC system, CPr-dG-d7 with any composition ratio of the (6S, 8S) form and the (6R, 8R) form could be used as an IS to quantitate the level of normal CPr-dG in real samples in which a variation in the constituent ratio of the diastereomers was commonly observed. Just to be sure, the concentration ratio of the (6S, 8S) form to the (6R, 8R) form of the deuterated CPr-dG standard was adjusted to 1.75:1 to be the same as that of the present natural CPr-dG standard preparation in the following discussions, including the preparation of a calibration curve for the quantitation of CPr-dG in samples.

Practically, any replacement of the deuterium atoms in CPr-dG-d7 as a stable isotope IS by hydrogen atoms in aqueous solutions within a realistic period is not permissible. An aqueous solution containing both the deuterated CPr-dG standard and the regular CPr-dG standard with concentrations of 1.0 × 10⁻⁹ M each was prepared, and the solution was subsequently stored at 4 °C for four weeks. Next, the peak areas of the solutions in the SRM chromatograms of m/z = 345.2 → 229.2 (CPr-dG-d7) and those of m/z = 338.1 → 222.1 (CPr-dG) in the present HILIC–ESI-MS/MS system were measured every seven days (three times each after the elapse of 0, 7, 14, 21, and 28 days). The ratio did not change substantially during the four weeks and was in the range of 0.85 ± 0.02, as indicated in Fig. 4. Additionally, the MS signal of m/z = 338.1...
identical to the base signal of normal CPr-dG did not appear in the MS spectra of another aqueous solution containing the deuterated CPr-dG standard of $10^{-9}$ M stored at 4 °C for four weeks.

A calibration curve of CPr-dG using the present HILIC–ESI-MS/MS system was made by using CPr-dG-$d_7$ ($1.0 \times 10^{-9}$ M) as the IS with sufficient linearity, $Y = 1.14X + 0.023$ ($r^2 = 0.998$, $n = 5$), in the concentration range from $1.0 \times 10^{-10}$ to $4.0 \times 10^{-9}$ M, where $X$ and $Y$ are the concentration of CPr-dG in the sample solutions and relative peak areas of CPr-dG to those of CPr-dG-$d_7$, respectively.

Conclusions

The deuterated CPr-dG consisting of mainly CPr-dG-$d_7$ was synthesized successfully using regular dG and acetaldehyde-$d_4$ and using D$_2$O as a reaction solvent. During storage in H$_2$O for four weeks, D-H change in CPr-dG-$d_7$ was not observed, and the stability necessary for a stable isotope IS was confirmed. Then, this IS was used to establish a calibration curve for CPr-dG with good linearity. Moreover, the isotopic IS of CPr-dG could be prepared from dG, acetaldehyde-$d_4$, and D$_2$O using the present method with much lower cost compared with CPr-dG-$^{15}N_5$ made from dG-$^{15}N_5$ and acetaldehyde. Although adenosine-$^{15}N_5$ has been employed as a universal IS to detect several kinds of nucleoside, including damaged ones, in our group, the present deuterated CPr-dG standard would enable us to correct both matrix effects in the ESI-MS/MS analysis of real samples and variations of CPr-dG recovery in the pretreatment of real samples, thus improving the reliability of its quantitation.

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References

1. J.-L. Fang and C. E. Vaca, Carcinogenesis, 1997, 18, 627.
2. M. Wang, E.J. McIntee, G. Cheng, Y. Sci, P.W. Villalta, and S.S. Hecht, Chem. Res. Toxicol., 2000, 13, 1149.
3. G. Obe and H. Ristow, Mutat. Res., 1977, 68, 291.
4. T. Matsuda, M. Kawanishi, T. Yage, S. Matsui, and H. Takebe, Nucleic Acid Res., 1998, 26, 1769.
5. T. Matsuda, H. Yabushita, R.T. Kanaly, S. Shibutani, and A. Yokoyama, Chem. Res. Toxicol., 2006, 19, 1374.
6. P. J. Brooks, M.-A Enoch, D.Goldman, T.-K. Li, A. Yokoyama, PLOS Med., 2009, 6, 258.
7. H. Murakami, R. Horiba, T. Iwata, Y. Miki, B. Uno, T. Sakai, K. Kaneko, Y. Ishihama, N. Teshima, and Y. Esaka, Talanta, 2018, 177, 12.
8. Internal Agency for Research on Cancer, IARC Monographs on the evaluation of carcinogenic risks to humans, Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide, 1999, 71, 319
9. Internal Agency for Research on Cancer, IARC Monographs on the evaluation of carcinogenic risks to humans. Alcohol consumption and ethyl carbamate, 2010, 96,
10. S. Inagaki, Y. Esaka, Y. Deyashiki, M. Sako, and M. Goto, J.Chromatogr.A., 2003, 987, 341

11. S.S. Hecht, Int J Cancer, 2012, 15, 2724.

12. S.S. Hecht, Chem.Res.Toxcol., 2017, 30, 367.

13. S. Zhang, P. W. Villalta, M. Wang, S. S. Hecht, Chem. Res. Toxicol., 2006, 19, 1386.

14. M. Wang, N. Tu, L. Chen, P.W. Villalta, J.B. Hochalter, and S.S. Hecht, Chem. Res. Toxicol., 2006, 19, 319.

15. M. Sako, I. Yaekura and Y. Deyashiki, Tetrahedron Letters, 2002, 43, 6701.

16. M. Sako and I. Yaekura, Tetrahedron, 2002, 58, 8413.

17. M. Sako, S. Inagaki, Y. Esaka and Y. Deyashiki, Bioorg. Med. Chem. Lett., 2003, 13, 3497.

18. S. Inagaki, Y. Esaka, M. Goto, Y. Deyashiki and M. Sako, Biol. Pharm. Bull., 2004, 27, 273.

19. M. Wang, E.J. McIntee, G. Cheng, Y. Sci, P.W. Villalta, and S.S. Hecht, Chem. Res. Toxicol., 2001, 14, 1025.

20. M. Wang, E.J. McIntee, G. Cheng, Y. Sci, P.W. Villalta, and S.S. Hecht, Chem. Res. Toxicol., 2001, 14, 423.

21. F.-L. Chung, S.S. Hecht, Cancer Res. 1983, 43, 1230.

22. J. McMurry, “Organic Chemistry”, 5th ed., Brooks/Cole, Pacific Grove, 1999, Chap. 22, 902.

23. A. Periat, I. Kohler, A. Bugey, S. Bieri, F. Versace, C. Staub and D. Guillarme, J. Chromatogr. A, 2014, 1356, 211.
Figure Captions

Fig. 1 Schematic mechanism of the formation of CPr-dG-\textit{d7} in D\textsubscript{2}O with arginine catalysis.\textsuperscript{15-18}

Fig. 2 MS spectra of the peak components in RP-HPLC of CPr-dG synthesized with acetaldehyde-\textit{d4} in H\textsubscript{2}O.

Fig. 3 Difference in MS spectra between the diastereomers of CPr-dG synthesized with acetaldehyde in H\textsubscript{2}O and those with acetaldehyde-\textit{d4} in D\textsubscript{2}O.

Fig. 4 Time course of the component ratio between CPr-dG-\textit{d7} and regular CPr-dG during storage in the presence of H\textsubscript{2}O at 10 °C.
Fig. 1 Schematic mechanism of the formation of CPr-dG-\(d_7\) in D\(_2\)O with arginine catalysis.\(^{15}\)
Fig. 2 MS spectra of the peak components in RP-HPLC of CPr-dG synthesized with acetaldehyde-$d_4$ in H$_2$O.
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