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The sphingolipid degradation product *trans*-2-hexadecenal forms adducts with DNA

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ABSTRACT

Sphingosine 1-phosphate, a bioactive signaling molecule with diverse cellular functions, is irreversibly degraded by the endoplasmic reticulum enzyme sphingosine 1-phosphate lyase, generating *trans*-2-hexadecenal and phosphoethanolamine. We recently demonstrated that *trans*-2-hexadecenal causes cytoskeletal reorganization, detachment, and apoptosis in multiple cell types via a JNK-dependent pathway. These findings and the known chemistry of related α,β-unsaturated aldehydes raise the possibility that *trans*-2-hexadecenal may interact with additional cellular components. In this study, we show that it reacts readily with deoxyguanosine and DNA to produce the diastereomeric cyclic 1,N^2-deoxyguanosine adducts 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-8*R*-hydroxy-6*R*-tridecylpyrimido[1,2-*a*]purine-10(3*H*)one and 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-8*S*-hydroxy-6*S*-tridecylpyrimido[1,2-*a*]purine-10(3*H*)one. Thus, our findings suggest that *trans*-2-hexadecenal produced endogenously by sphingosine 1-phosphate lyase can react directly with DNA forming aldehyde-derived DNA adducts with potentially mutagenic consequences.

1. Introduction

Sphingosine 1-phosphate lyase (SPL) catalyzes the conversion of sphingosine 1-phosphate (**1**) to *trans*-2-hexadecenal (**2**) and phosphoethanolamine (**3**) (Scheme 1) [1]. The roles of **1** in cell and animal physiology have been well described [2], but the biological consequences and fate of metabolic products of **1** such as **2** are not well understood. We have previously shown that **2** causes cytoskeletal reorganization, detachment, and apoptosis in multiple cell types via a JNK-dependent pathway, indicating that it may interact with multiple cellular components including DNA [3]. Previous studies have shown that α,β-unsaturated aldehydes such as acrolein, crotonaldehyde (2-butenal), and 4-hydroxy-2-nonenal react readily with deoxyguanosine (dG, **4**) and DNA to produce cyclic 1,N^2-propanodeoxyguanosine adducts with a variety of mutagenic properties [4–10]. Therefore, we hypothesized that **2** would undergo a similar reaction (Scheme 1), potentially producing an adduct such as **5**, which could be central to some of the physiologic effects of **2**. We tested this hypothesis in the study reported here.

2. Materials and methods

2.1. HPLC systems

System 1 consisted of a Waters Corp. model 600 system controller, two model 501 pumps, a model 440 UV detector (254 nm), and an Agilent Technologies 1100 series auto-sampler. A Luna $C_{18}(2)$ reversed-phase column (250 × 4.6 mm, 5 μm; Phenomenex) was used for the separation. Elution was performed with a linear gradient from 85% 15 mM NH_4OAc in CH_3OH to 100% CH_3OH over 30 min and held for 20 min. The flow rate was 0.7 ml/min.

System 2 used an Agilent Technologies 1100 capillary HPLC system equipped with a 5 μm, 150 × 0.5 mm ZorbaxSB-C18 column eluted at 8 μl/min with 70% 15 mM NH_4OAc in CH_3OH to 99% CH_3OH in 30 min and held for 15 min.

System 3 was the same as System 2 except that elution was with 85% 15 mM NH_4OAc in CH_3OH to 95% CH_3OH in 30 min and held for an additional 25 min.

2.2. NMR spectra

These were recorded in DMSO-d_6 using a Bruker Biospin (Billerica, MA) spectrometer operating at 850 MHz. Resonance assignments were made based on ^1H–^1H COSY and ^1H–^{13}C HMBC and HSQC experiments acquired at 850 MHz.

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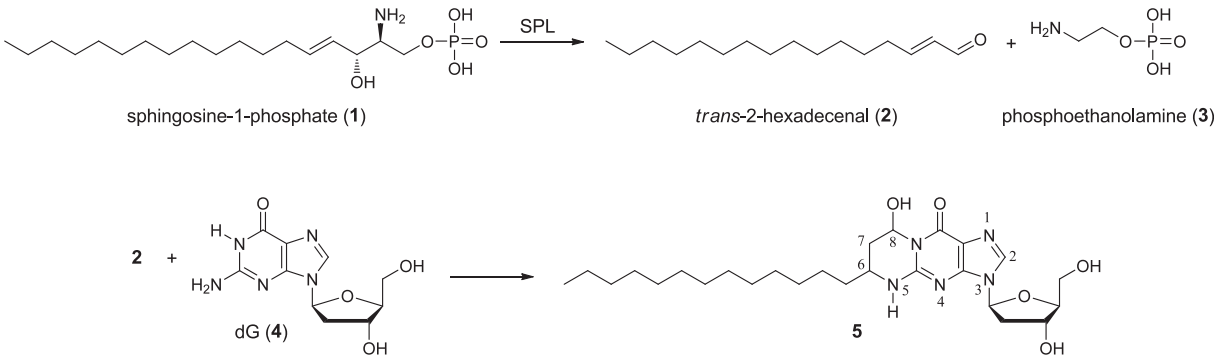


Scheme 1. Conversion of sphingosine 1-phosphate (**1**) to *trans*-2-hexadecenal (**2**) and reaction of **2** with dG (**4**) to form adduct **5**.

2.3. trans-2-Hexadecenal (2)

This compound was prepared as described previously [11].

2.4. Reaction of 2 with dG

trans-2-Hexadecenal (**2**, 4.7 mg, 20 μmol) was allowed to react with dG (**4**, 20 mg, 0.074 mmol) in 5 ml of 0.10 M phosphate buffer, pH 7.0, at 50 °C for 72 h. Adduct **5**, retention time 34 min, was purified using HPLC system 1 and analyzed by liquid chromatography-electrospray ionization-tandem mass spectrometry-selected reaction monitoring (LC–ESI-MS/MS-SRM) using system 2 and a Thermo TSQ Quantum Discovery MAX instrument, in the positive ion mode with N_2 as the nebulizing and drying gas. MS parameters were set as follows: spray voltage, 4 Kv; scan width, 0.4 amu; sheath gas pressure, 20; capillary temperature 250 °C; collision energy 20 V (for *m/z* 390–372 and *m/z* 390–346) and 28 V for other ions; and tube lens offset, 140 V. Data were acquired and processed by Xcalibur software version 1.4. The mass transitions monitored were *m/z* 506 → 390, *m/z* 390 → 346, *m/z* 390 → 372, *m/z* 390 → 190, and *m/z* 390 → 152.

To enable collection of sufficient material for NMR analysis, a larger scale reaction with 56.4 mg (0.24 mmol) of **2** and 240 mg (0.89 mmol) of **4** was performed for 72 h at 50 °C. The reaction was carried out in 25 ml of 25 mM potassium phosphate buffer, pH 9.0, since Michael addition of dG to α,β-unsaturated aldehydes appears to proceed at higher rates at alkaline pH [9]. Adduct **5** was collected using system 1 (<0.5% yield). ^{1}H NMR (850 MHz, DMSO-d_6) δ 7.912 and 7.913 (2s, 1H, C2-H), 7.82 (d, 1H, N5-H), 6.65 (m, 1H, C8-OH), 6.2 (s, 1H, C8-H), 6.1 (1H, t, J = 6.3 Hz, 1′-H), 5.28 (m, 1H, 3′-OH), 4.92 (m, 1H, 5′-OH), 4.36 (br s, 1H, 3′-H), 3.79 (m, 1H, 4′-H), 3.54 (m, 1H, C6-H), 3.5–3.49 (m, 2H, 5′-H_2), 2.5 (m, 1H, 2′-H), 2.18 (m, 1H, 2′-H), 2.06 (1H, d, J = 14 Hz, C7H), 1.4 (m, 3H, C7-H + C9-H_2), 1.2 (br s, 20H, alkyl chain CH_2), 0.87 (t, 3H, CH_3); ^{13}C NMR (850 MHz, DMSO-d_6) exocyclic carbons 44.81(C6), 34.56 (C7), 69.77 (C8); guanine carbons 135.75 (C2), 156.08 (C = O); alkyl chain 31.77 (CH_2-Gua), 14.45 (CH_3); deoxyribose 62.14 (C-5′), 87.92 (C-4′), 71.18 (C-3′), and 82.71 (C-1′); UV (90% CH_3OH/10% 15 mM NH_4OAc) λ_{max} 204, 254, 275 (sh) nm: ESI-HRMS (*m/z*) $[M + H]^+$ calcd for $C_{26}H_{43}N_5O_5Na$, 528.3156; found 528.3169.

2.5. Acid hydrolysis of 5

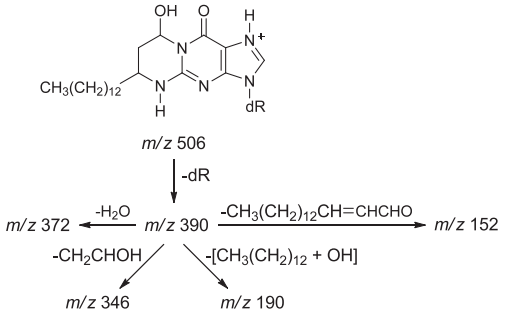
In brief, 50 μg of **5** was dissolved in 1 ml of HCl (0.10 N) and the mixture was heated at 90 °C for 60 min. The mixture was cooled, neutralized with 1 N NaOH, and applied to a Strata-X polymeric sorbent solid-phase extraction cartridge (33 μm, 30 mg/1 ml, Phenomenex). The cartridge was sequentially eluted with 1 ml of H_2O, 1 ml of 10% methanol, and 2 ml of methanol, and then the product was worked up as described (9). The resulting G adduct was analyzed by LC–ESI-MS-MS-SRM using system 2, positive ion mode *m/z* 390 → *m/z* 346, 390 → *m/z* 372, *m/z* 390 → *m/z* 190, and *m/z* 390 → *m/z* 152.

2.6. Reaction of 2 with DNA

trans-2-Hexadecenal (**2**, 2.4 mg, 10 μmol) was allowed to react with calf thymus DNA (10 mg) in 5 ml of 0.10 M phosphate buffer, pH 7.0, for 24 h at 37 °C. The DNA was precipitated by addition of ethanol and washed with 70% and 95% ethanol. After the DNA (0.5 mg) was dissolved in 1 ml of Tris buffer, pH 7.0, enzyme hydrolysis was carried out as described [12]. The hydrolysate was loaded onto a Strata-X polymeric sorbent solid-phase extraction cartridge (33 μm, 30 mg/1 ml) that was previously activated with 1 ml of CH_3OH and 1 ml of H_2O. The cartridge was washed with 2 ml of H_2O, and the analyte was eluted with 2 ml of 100% CH_3OH. The eluant was collected in a 2 ml silanized vial, and the solvents were removed on a Speedvac. The residue was taken up in 300 μl of CH_3OH, transferred to an insert vial, and concentrated to dryness. The residue was dissolved in 20 μl of 70% aqueous NH_4OAc in CH_3OH, and 8 μl was analyzed by LC–ESI-MS/MS-SRM using system 3, positive ion mode, *m/z* 506 → 390. Incubation of **1** with calf thymus DNA for 72 h gave a higher yield of **5**.

2.7. Acid hydrolysis of the calf thymus DNA adduct

In brief, 0.5 mg of DNA from reaction of **2** and calf thymus DNA was dissolved in 1 ml of HCl (0.10 N), heated at 90 °C for 60 min, and then was worked up as described under "Acid hydrolysis of **5**" (Section 2.5). The resulting G adduct was analyzed by LC–ESI-MS-MS-SRM using system 2 (*m/z* 390 → 346, *m/z* 390 → 372, *m/z* 390 → 190, and *m/z* 390 → 152).



Scheme 2. Rationalization of the MS fragmentation of **5**, as seen in Fig. 1. dR = deoxyribose.

3. Results and discussion

trans-2-Hexadecenal (**2**) was allowed to react with dG (**4**), and the products were analyzed by LC–ESI-MS/MS-SRM. The transitions monitored were *m/z* 506 → 390 $[BH]^+$, *m/z* 390 → 346 $[BH–CH_2CHOH]^+$, *m/z* 390 → 372 $[BH–H_2O]^+$, *m/z* 390 → 190 $[BH–(CH_3(CH_2)_{12}+OH)]^+$, and *m/z* 390 → 152 $[GH]^+$ (Scheme 2). As shown in Fig. 1, all of these transitions were observed and are consistent with the expected product, **5**.

The UV spectrum and the 850 MHz ^1H NMR and ^{13}C NMR spectra of **5** are completely consistent with those of other exocyclic 1,N^2-propano-dG adducts produced by the reaction of α,β-unsaturated aldehydes with dG [9,13,14] and all of the assignments were confirmed by $^1H–^1H$ COSY and $^1H–^{13}C$ HMBC and HSQC experiments (data not shown).

Previous studies demonstrated that adducts such as **5** are mixtures of 6*S*, 8*S*-and 6*R*, 8*R*-diastereomers in which the alkyl and hydroxy groups are trans to each other [4–10]. Importantly, the 850 MHz ^1H NMR data obtained in this study allowed us to observe 2 singlets at 7.912 and 7.913 ppm, corresponding to the protons at the 2-position of each of the two diastereomers. Observation of these distinct signals has not been reported previously in studies using lower field strength NMR instruments.

Acid hydrolysis of adduct **5** provided the corresponding G adduct, as determined by LC–ESI-MS/MS-SRM analysis for the transitions *m/z* 390 → 346, *m/z* 390 → 372, *m/z* 390 → 190, and *m/z* 390 → 152. The results are virtually identical to those shown in Fig. 1, except that the retention time was 39.6 min. All fragments are consistent with Scheme 2, starting with the G adduct.

Reaction of **2** with calf thymus DNA, followed by enzymatic hydrolysis and LC–ESI-MS/MS-SRM analysis, produced the chromatogram illustrated in Fig. 2, which shows the presence of **5** in the hydrolysate. Acid hydrolysis of this DNA produced the G adduct, which was identified by LC–ESI-MS/MS-SRM analysis and co-injection with the standard G adduct.

Collectively, these results establish the structures of the products of the reaction of **2** with dG or DNA as a mixture of 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-8*R*-hydroxy-6*R*-tridecylpyrimido[1,2-*a*]purine-10(3*H*)one and 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-8*S*-hydroxy-6*S*-tridecylpyrimido[1,2-*a*]purine-10(3*H*)one (**5**). While these are the expected products based on literature precedent [4–10], we note that there are seven more carbons in the alkyl side chain than any previously reported structures of this type. These amphilic adducts may have some special properties because of the combination of lipophilic and hydrophilic residues in the same molecule.

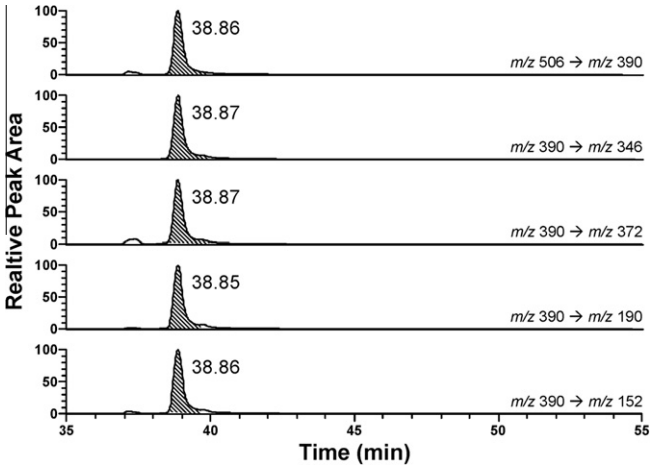


Fig. 1. LC–ESI-MS/MS-SRM analysis of **5** formed in the reaction of *trans*-2-hexadecenal (**2**) with dG (**4**). See Scheme 1 for structures and Scheme 2 for MS fragmentation.

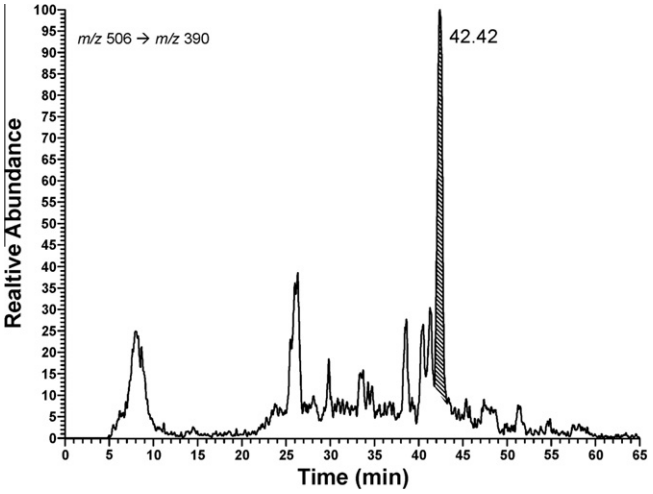


Fig. 2. LC–ESI-MS/MS-SRM analysis of an enzymatic hydrolysate of calf thymus DNA that had been reacted with **2**. The shaded peak is adduct **5**.

The results of this study support our hypothesis that **2**, an endogenous α,β-unsaturated aldehyde produced by the action of SPL on **1**, as shown in Scheme 1, reacts with DNA and may lead to potentially mutagenic consequences or perhaps trigger a previously unrecognized DNA damage response. A variety of mutagenic properties of structurally related dG adducts derived from acrolein, crotonaldehyde, and 4-hydroxynonenal have been observed, and the ultimate biological properties of these adducts are influenced by DNA sequence context effects, chromatin condensation, DNA repair mechanisms, cross-linking, and other factors [10]. It will be important to determine the biological properties of adduct **5** and analyze human DNA samples for its occurrence, as many previous studies have shown the presence of related endogenous DNA adducts such as those formed from acrolein and crotonaldehyde in DNA from human liver, lung, leukocytes, and other tissues [7,15–19].

Acknowledgments

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References

[1] B. Oskouian, J. Saba, Sphingosine-1-phosphate metabolism and intestinal tumorigenesis: lipid signaling strikes again, Cell Cycle 6 (2007) 522–527.
[2] A. Kumar, J.D. Saba, Lyase to live by: sphingosine phosphate lyase as a therapeutic target, Expert Opin. Ther. Targets 13 (2009) 1013–1025.
[3] A. Kumar, H.S. Byun, R. Bittman, J.D. Saba, The sphingolipid degradation product *trans*-2-hexadecenal induces cytoskeletal reorganization and apoptosis in a JNK-dependent manner, Cell. Signal 23 (2011) 1144–1152.
[4] F.L. Chung, R. Young, S.S. Hecht, Formation of cyclic 1,N^2-propanodeoxyguanosine adducts in DNA upon reaction with acrolein or crotonaldehyde, Cancer Res. 44 (1984) 990–995.
[5] F.L. Chung, K.R. Roy, S.S. Hecht, A study of reactions of α,β-unsaturated carbonyl compounds with deoxyguanosine, J. Org. Chem. 53 (1988) 14–17.
[6] R.G. Nath, J.E. Ocando, F.L. Chung, Detection of 1,N^2-propanodeoxyguanosine adducts as potential endogenous DNA lesions in rodent and human tissues, Cancer Res. 56 (1996) 452–456.
[7] F.L. Chung, L. Zhang, J.E. Ocando, R.G. Nath, Role of 1,N^2-propanodeoxyguanosine adducts as endogenous DNA lesions in rodents and humans, in: B. Singer, H. Bartsch (Eds.), Exocyclic DNA Adducts in Mutagenesis

and Carcinogenesis, International Agency for Research on Cancer, Lyon, France, pp. 45–54.
[8] F.L. Chung, R.G. Nath, J. Ocando, A. Nishikawa, L. Zhang, Deoxyguanosine adducts of t-4-hydroxy-2-nonenal are endogenous DNA lesions in rodents and humans: detection and potential sources, Cancer Res. 60 (2000) 1507–1511.
[9] C.K. Winter, H.J. Segall, W.F. Haddon, Formation of cyclic adducts of deoxyguanosine with the aldehydes *trans*-4-hydroxy-2-hexenal and *trans*-4-hydroxy-2-nonenal *in vitro*, Cancer Res. 46 (1986) 5682–5686.
[10] I.G. Minko, I.D. Kozekov, T.M. Harris, C.J. Rizzo, R.S. Lloyd, M.P. Stone, Chemistry and biology of DNA containing 1, N(2)-deoxyguanosine adducts of the alpha, beta-unsaturated aldehydes acrolein, crotonaldehyde, and 4-hydroxynonenal, Chem. Res. Toxicol. 22 (2009) 759–778.
[11] W.J. Baumann, H.H. Schmid, H.K. Mangold, Oxidative cleavage of lipids with sodium metaperiodate in pyridine, J. Lipid Res. 10 (1969) 132–133.
[12] S. Zhang, M. Wang, P.W. Villalta, B.R. Lindgren, P. Upadhyaya, Y. Lao, S.S. Hecht, Analysis of pyridyloxobutyl and pyridylhydroxybutyl DNA adducts in extrahepatic tissues of F344 rats treated chronically with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and enantiomers of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, Chem. Res. Toxicol. 22 (2009) 926–936.
[13] F.L. Chung, S.S. Hecht, Formation of cyclic 1,N^2-adducts by reaction of deoxyguanosine with α-acetoxy-*N*-nitrosopyrrolidine, 4-(carbethoxynitrosamino)butanal, or crotonaldehyde, Cancer Res. 43 (1983) 1230–1235.
[14] E. Eder, C. Hoffman, Identification and characterization of deoxyguanosine adducts of mutagenic β-alkyl-substituted acrolein congeners, Chem. Res. Toxicol. 6 (1993) 486–494.
[15] F.L. Chung, H.J.C. Chen, R.G. Nath, Lipid peroxidation as a potential source for the formation of exocyclic DNA adducts, Carcinogenesis 17 (1996) 2105–2111.
[16] A. Emami, M. Dyba, A.K. Cheema, J. Pan, R.G. Nath, F.L. Chung, Detection of the acrolein-derived cyclic DNA adduct by a quantitative ^{32}P-postlabeling/solid-phase extraction/HPLC method: blocking its artifact formation with glutathione, Anal. Biochem. 374 (2008) 163–172.
[17] S. Zhang, P.W. Villalta, M. Wang, S.S. Hecht, Analysis of crotonaldehyde- and acetaldehyde-derived 1,N^2-propanodeoxyguanosine adducts in DNA from human tissues using liquid chromatography-electrospray ionization-tandem mass spectrometry, Chem. Res. Toxicol. 19 (2006) 1386–1392.
[18] S. Zhang, P.W. Villalta, M. Wang, S.S. Hecht, Detection and quantitation of acrolein-derived 1,N^2-propanodeoxyguanosine adducts in human lung by liquid chromatography-electrospray ionization-tandem mass spectrometry, Chem. Res. Toxicol. 20 (2007) 565–571.
[19] S. Zhang, S. Balbo, M. Wang, S.S. Hecht, Analysis of acrolein-derived 1,N^2-propanodeoxyguanosine adducts in human leukocyte DNA from smokers and nonsmokers, Chem. Res. Toxicol. 24 (2011) 119–124.