Available online at www.sciencedirect.com
SCIENCE DIRECT
Analytical Biochemistry 348 (2006) 15–23
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

A solid-phase extraction/high-performance liquid chromatography-based ^{32}P-postlabeling method for detection of cyclic 1,N^2-propanodeoxyguanosine adducts derived from enals

Jishen Pan [a], Warren Davis [b], Neil Trushin [c], Shantu Amin [c], Raghu G. Nath [a], Norman Salem Jr. [d], Fung-Lung Chung [a,*]

[a] *Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC 20057, USA*
[b] *Roswell Park Cancer Institute, Buffalo, NY 14263, USA*
[c] *College of Medicine, The Pennsylvania State University, Hershey, PA 17033, USA*
[d] *National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852, USA*

Received 12 May 2005
Available online 27 October 2005

Abstract

The cyclic 1,N^2-propanodeoxyguanosine (PdG) adducts are Michael addition products from reactions of deoxyguanosine (dG) with enals, including acrolein (Acr), crotonaldehyde (Cro), pentenal (Pen), heptenal (Hep), and 4-hydroxy-2-nonenal (HNE). Although this is a general reaction, only the PdG adducts derived from Acr, Cro, and HNE have been detected in vivo as endogenous DNA lesions. Our previous in vitro study demonstrated that PdG adducts of Acr, Cro, and Pen are predominantly derived from oxidation of ω-3 polyunsaturated fatty acids (PUFAs), whereas the long-chain Hep and HNE adducts are from ω-6 PUFAs. PdG adducts are important because they represent a new class of endogenous promutagenic DNA lesions with potential roles in carcinogenesis. Earlier, we developed a ^{32}P-postlabeling method for detecting PdG adducts from Acr and Cro and a modified method for the long-chain HNE adducts. Both methods require multiple high-performance liquid chromatography steps and, in some cases, time-consuming thin-layer chromatography for purification. There is a lack of a single, versatile, and efficient method for simultaneous detection of all five enal-derived PdG adducts. In this paper, we report an improved ^{32}P-postlabeling method which permits detection of Acr, Cro, Pen, Hep, and HNE adducts in a single DNA sample. This method relies on solid-phase extraction for adduct enrichment before and after ^{32}P-labeling; all five PdG adducts were converted to the ring-opened derivatives for confirmation of identities and quantification. The method was validated using the synthetic adducts and enal-modified DNA and was finally applied to rat liver DNA and rat liver DNA samples spiked with different amount of standards. The detection limit was determined to be as low as 0.5 fmol in 80 μg DNA, corresponding to 9 adducts/10^9 dG.

Keywords: 1,N^2-propanodG; Enal-derived cyclic adducts; Aldehyde-DNA adducts; Acrolein; 4-Hydroxy-2-nonenal; Cyclic deoxyguanosine adducts; ^{32}P-postlabeling; High-performance liquid chromatography; Solid-phase extraction

α,β-Unsaturated aldehydes or enals are a family of reactive compounds amenable to undergoing Michael addition with cellular nucleophiles, such as DNA and proteins. The simplest and most abundant enals are acrolein (Acr)[1] and crotonaldehyde (Cro), found ubiquitously in the environment generated through fossil fuel combustion and cigarette smoking [1,2]. Lipid peroxidation is a major pathway for endogenous formation of enals, including

* Corresponding author. Fax: +1 202 687 1068.
E-mail address: flc6@georgetown.edu (F.-L. Chung).

[1] *Abbreviations used:* Acr, acrolein; Cro, crotonaldehyde; dA, deoxyadenosine; dG, deoxyguanosine; dH_2O, deionized water; Hep, *trans*-2-heptenal; HNE, *trans*-4-hydroxy-2-nonenal; HPLC, high-performance liquid chromatography; Pen, *trans*-2-pentenal; PdG, 1,N^2-propanodeoxyguanosine; T4 PNK, T4 polynucleotide kinase; SPE, solid-phase extraction; TLC, thin-layer chromatography.

0003-2697/$ - see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.ab.2005.10.011

Acr, Cro, pentenal (Pen), hexenal, heptenal (Hep), and *t*-4-hydroxynonenal (HNE) [3–5]. Enals are cytotoxic and genotoxic by causing DNA strand breaks, DNA–protein cross-linking, and protein and DNA adducts [6,7].

Enals react with deoxyguanosine (dG) in DNA yielding isomeric 1,N^2-propanodeoxyguanosine (PdG) adducts featuring an extended and saturated six-member ring shown in Fig. 1 [7–10]. For the isomeric Acr adducts, we focus on the Acr-dG in Fig. 1, because, for reasons yet to be determined, this is the predominant regio-isomer detected in vivo [11]. Other known cyclic adducts with unsaturated six-member rings (propeno) or five-member rings (etheno) can be formed from reactions with malondialdehyde or the epoxides of enals, respectively [7,12,13]. Various methods using immunoassay, liquid chromatography–mass spectroscopy (LC–MS), gas chromatography–mass spectroscopy (GC–MS), and ^{32}P-postlabeling have been developed to detect these adducts in vitro and in vivo [11,14–17]. Among these methods, the ^{32}P-postlabeling is the most sensitive and has been used extensively for the detection of cyclic PdG adducts in vivo [11,17,18]. The discovery of the enal-derived cyclic adducts in tissues of rodents and humans as endogenous DNA lesions by this method has raised questions about their roles in mutagenesis and carcinogenesis [18,19].

Earlier, we reported a ^{32}P-postlabeling assay in conjunction with HPLC and thin-layer chromatography (TLC) specifically for the detection of Acr- and Cro-derived PdG adducts [11]. This method was later modified for HNE-dG adducts possessing a long alkyl chain [20]. Both methods, while similar, have to be used independently for the detection of Acr and Cro adducts and HNE adducts in vivo. In addition to the high sensitivity, a major advantage of these methods is their ability to detect the diastereomers of Acr-, Cro-, and HNE-dG adducts, but they suffer from low recoveries and poor efficiency as a result of multiple HPLC and time-consuming TLC steps and their utility is limited to detecting either Acr and Cro or HNE adducts. The main purpose of this study was to develop a more versatile and efficient method for simultaneous detection of all five PdG adducts from enals with varying alkyl chain lengths from Acr to HNE by incorporating solid-phase extraction (SPE) to improve its recovery and quantification.

Materials and methods

Chemicals

Acr, Cro, Pen, Hep, calf thymus DNA, micrococcal nuclease, nuclease P1, and apyrase were obtained from Sigma–Aldrich (St. Louis, MO), and HNE was synthesized by a previously described method [21]. Spleen phosphodiesterase was from Worthington Biochemical (Lakewood, NJ), and [γ-^{32}P]ATP and T4 polynucleotide kinase (T4 PNK) were from Amersham (Piscataway, NJ). All other reagents, unless otherwise stated, were from Sigma–Aldrich and Fisher Chemical (Fair Lawn, NJ). The 3′-monophosphates of Acr-, Cro-, Pen-, Hep-, and HNE-dG were prepared as previously described, and the identities of these adduct standards were established by their UV spectra and mass spectroscopy [22–24]. The 3′,5′-bisphosphates of these adducts were prepared by incubating the 3′-monophosphates of these adducts with T4 PNK and ATP as previously reported [22,23].

HPLC systems

The HPLC system consisted of two LC-10AD VP pumps, an SCL-10A VP controller, and an SPD-M10A VP photodiode array detector (Shimadzu, Kyoto, Japan) with a Prodigy ODS(3) C18 reverse-phase column (5 μm, 250 mm × 4.6 mm) from Phenomenex (Torrance, CA). The solvent systems used were as follows: System 1, A: 50 mM NaH_2PO_4 (pH 5.8), B: 50% methanol with 100% A for 20 min followed by 0→80% B in 80 min at 0.6 ml/min. System 2, A: 25 mM triethylamine phosphate (pH

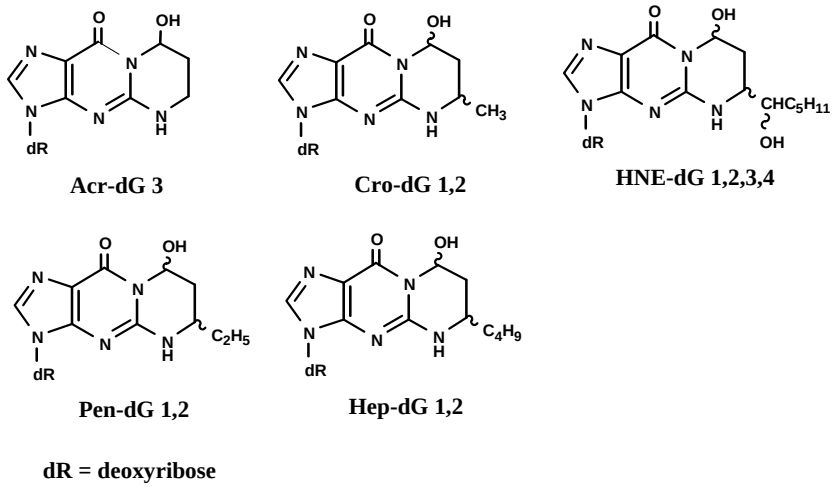

Fig. 1. Structures of adducts of 1,N^2-propanodeoxyguanosine.