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Short Communication

Release of 2-aminofluorene from N-(deoxyguanosin-8-yl)-2-aminofluorene by hydrazinolysis

JOTHI BAKTHAVACHALAM[a], SAMY ABDEL-BAKY and ROGER W. GIESE*
Department of Medicinal Chemistry in the College of Pharmacy and Allied Health Professions, and Barnett Institute of Chemical Analysis and Materials Science, Northeastern University, Boston, MA 02115 (U.S.A.)
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ABSTRACT

High temperature (160°C) hydrazinolysis is demonstrated to be a useful chemical transformation reaction for releasing an aminopolyaromatic hydrocarbon, 2-aminofluorene, from a parent DNA adduct, N-(deoxyguanosin-8-yl)-2-aminofluorene.

INTRODUCTION

Inherent structural changes are sometimes made in an analyte as part of sample pretreatment in order to improve its separation and/or detection properties in an analytical procedure. This general strategy can be called "chemical transformation". Examples are the oxidation of cholesterol [1], the deamination of N7-(2-hydroxyethyl)guanine [2], the acidic reduction of tryptophan [3] and the alkaline reduction of thymine glycol [4] in methods used to quantify these substances.

We are interested in broadening the usefulness of gas chromatography (GC) with electron capture negative ion mass spectrometry (ECNI-MS) for the sensitive detection of DNA adducts by using chemical transformation as part of sample pretreatment. DNA adducts are trace analytes which constitute the covalent damage to *in vivo* DNA when an animal or human is exposed to toxic chemical or physical conditions. Conventional derivatization reactions are adequate to convert many small DNA adducts (*e.g.*, damage to DNA by methylating agents) into products which can be detected by GC–ECNI-MS [5]. However, for large DNA adducts (*e.g.*, damage to DNA by polyaromatic hydrocarbons), chemical transformation is needed because the low volatility and thermal stability of such adducts for GC purposes cannot be adequately overcome by conventional derivatization.

[a] Present address: Polaroid and Behringwerke Diagnostic Systems, Westwood, MA 02090, U.S.A.

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Here we introduce a new reaction, in terms of the substrate and conditions, which achieves the release of 2-aminofluorene from the polyaromatic hydrocarbon DNA adduct, N-(deoxyguanosin-8-yl)-2-aminofluorene. The overall goal is to establish a method by which this adduct can be measured using GC–ECNI-MS. In the proposed method, 2-aminofluorene is released from the adduct by this chemical transformation reaction, converted into an electrophoric derivative, and detected by GC–ECNI-MS.

Polyaromatic hydrocarbon DNA adducts can be measured by immunoassay [6], ^{32}P-post labeling [7] and fluorescence [8] procedures. These methods do not always provide sufficient scope, sensitivity and precision. For example, different values can be obtained when they are applied to the measurement of a given DNA adduct, or class of adducts, in the same samples [9,10]. Potentially some improvements over existing methods for the measurement of polyaromatic hydrocarbon DNA adducts can be achieved by using GC–ECNI-MS.

EXPERIMENTAL

Reagents

Anhydrous hydrazine (Aldrich, Milwaukee, WI, U.S.A.) was treated with 20% (w/w) of KOH overnight, followed by distillation under anhydrous conditions. The fraction that distilled at 110°C was collected. 2-Aminofluorene (98%) was from Aldrich. High-performance liquid chromatography (HPLC) solvents were from Burdick & Jackson (American Scientific, Boston, MA, U.S.A.). N-(Deoxyguanosin-8-yl)-2-aminofluorene (dGAF) and N-(deoxyguanosin-8-yl)-2-acetylaminofluorene were kindly provided by Dr. F. A. Beland (National Center for Toxicological Research, Jefferson, AR, U.S.A.).

Hydrazinolysis procedure

A methanolic solution (5 μl from a more concentrated stock solution) of dGAF (116.5 ng) was evaporated in a 1 ml ampule, followed by the addition of 20 μl of anhydrous hydrazine. The ampule was flame-sealed and heated at 180°C for 24 h in a Reacti-Block aluminium block E-1 (13 ampule capacity; Pierce, Rockford, IL, U.S.A.). This heating was achieved by placing the block in a Pyrex crystallizing dish (50 × 75 mm I.D.) which had been filled to a depth of 3.5 cm with silicone oil. Each hole in the heating block had been filled to a depth of 1.5 cm with sea sand, in order to heat primarily the bottom of the ampule. The ampule was cooled to room temperature, its top removed, and the excess reagent was evaporated to dryness using a Speed-Vac. After the addition of 100 μl of acetonitrile–water (2:1, v/v) 10 μl was injected into an HPLC system.

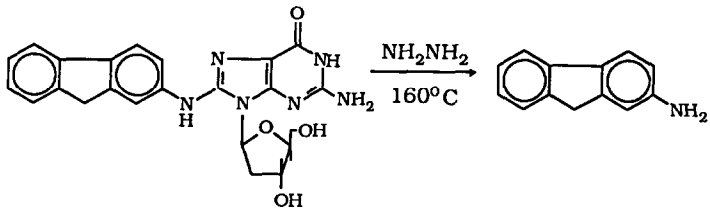
RESULTS AND DISCUSSION

Previously we optimized the derivatization of aminopolyaromatic hydrocarbons (amino-PAHs), such as 2-aminofluorene, for the purpose of detecting such compounds by GC–ECNI-MS [11]. Electrophoric derivatives were formed, and the most promising one was a pentafluorobenzylidene derivative. In order to use this methodology to measure corresponding DNA adducts, in which an amino-PAH

moiety is covalently attached to DNA, it is necessary to first liberate the amino-PAH. Towards this goal, we subjected the DNA adduct, N-(deoxyguanosin-8-yl)-2-aminofluorene, to a wide range of acidic and basic hydrolysis conditions (data not shown). While the desired product, 2-aminofluorene (2-AF), was always observed by HPLC, the yield never rose much above 10%.

A somewhat higher yield of 2-AF was formed (20%) when the parent adduct (*e.g.*, 10 μg) was subjected to hydrazinolysis under conventional conditions (100°C, 24 h). Increasing the reaction temperature (but not the time) up to 200°C increased the yield to 65%. The recovery of the product itself, 2-AF, subjected to hydrazinolysis under these conditions was 86 $\pm$ 3%. These collective observations suggested that the lower yields of 2-AF from the adduct at lower temperature were not due to instability of the 2-AF product under the reaction conditions. Instead a higher temperature apparently favored the formation of product, 2-AF, over unknown side products.

Thus we have achieved the following new chemical transformation.



Changing the reaction vessel from a capped vial to a flame-sealed ampule improved the precision of the yield by overcoming variation from one vial to another in leakage of the hydrazine through the vial seal. This did not present a safety problem because small volumes (20 μl) of hydrazine were employed.

The yield of 2-AF from the adduct was about 10% higher when other forms or derivatives of hydrazine were tested: hydrazine hydrochloride, carbohydrazide, hydroxyethyl-hydrazine, *p*-hydrazinobenzoic acid and 2-hydrazino-2-imidazoline hydrobromide. However, the increase in yield was small with these other reagents, and hydrazine can be removed at the end of the reaction by evaporation. (In our method, we either do this, or remove the hydrazine by HPLC after dilution of the reaction mixture with water and methanol.) Thus we selected hydrazine for additional study.

In the optimized, overall procedure that we developed, the parent DNA adduct is subjected to hydrazinolysis at 160°C for 24 h. The reaction shows promise for being applied to a trace amount of analyte. For example, the yield of 2-AF (by HPLC) starting from 115 ng of the adduct is 62 $\pm$ 2%. The related adduct, N-(deoxyguanosin-8-yl)-2-acetylaminofluorene, also forms 2-AF in the same yield. As shown in Fig. 1, aside from non-retained, polar compounds, analysis of the crude reaction mixture by HPLC shows only a single peak, which is the product.

We have also extended our earlier work [11] on the detection of 2-AF by GC–ECNI-MS. As shown in Fig. 2, 10 fg (25 amol) of the pentafluorobenzylidine derivative of the compound can be detected by this technique. DNA adducts, such as N-(deoxyguanosin-8-yl)-2-aminofluorene, may need to be measured in this range when biological samples are encountered. Thus, in our future work, we need to

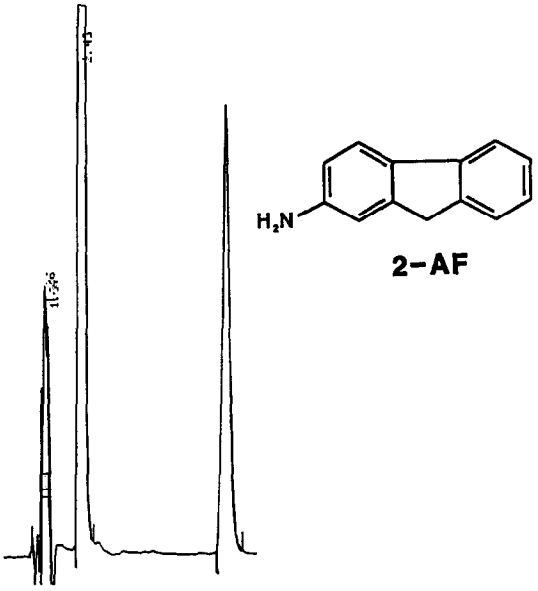


Fig. 1. HPLC separation of the hydrazinolysis reaction. N-(Deoxyguanosin-8-yl)-2-aminofluorene (5 μg) was subjected to hydrazinolysis in 150 μl of hydrazine. The reaction mixture was treated with 100 μl of water and 150 μl of methanol, then 10 μl of this solution were injected into the HPLC. Column: Brownlee (Rainin Instruments, Woburn, MA, U.S.A.) analytical cartridge, RP-8 reversed-phase, (10 cm × 4.6 mm I.D.), 5 μm particle size. Mobile phase: acetonitrile–10 m*M* potassium dihydrogen phosphate adjusted to pH 6.6 with 5 m*M* triethylamine (45:55, v/v) at 1 ml/min. Detection at 280 nm. Retention time 6.8 min.

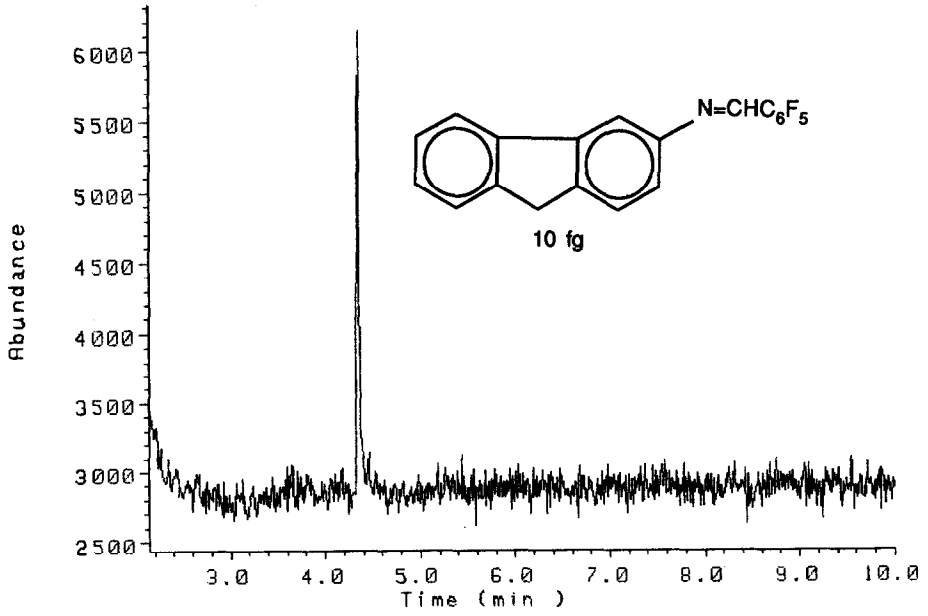


Fig. 2. Detection of N-pentafluorobenzylidenyl-2-aminofluorene by GC–ECNI-MS. Sample: 1 μl of toluene containing 10 fg of analyte. Instrument: Hewlett-Packard (Andover, MA, U.S.A.) Model 5890/5988A. Injection: splitless mode with splitless valve open after 0.8 min. Column: 12 m × 0.2 mm I.D., 0.33 μm film, Ultra-1 (Hewlett-Packard) fused-silica capillary, with 20 p.s.i. helium carrier gas. Conditions: 2 Torr of methane in the source; electron energy 240 eV, source temperature 200°C, emission current 300 μA, interface between GC and MS at 280°C, initial GC oven temperature 100°C then ramp to 280°C at 60°C/min and hold for 5 min. Signal-to-noise ratio 40.

combine and extend the steps presented here to achieve the detection of this adduct in such samples. The effectiveness of the hydrazinolysis reaction on other adducts of this type will also be studied. It is encouraging that others have found the reaction to similarly transform a corresponding 4-aminobiphenyl DNA adduct [12].

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REFERENCES

1 E. A. Stein, in N. W. Tietz (Editor), *Textbook of Clinical Chemistry*, Saunders, Philadelphia, PA, 1986, p. 882.
2 K. Allam, M. Saha and R. W. Giese, *J. Chromatogr.*, 499 (1990) 571.
3 W. S. D. Wong, D. T. Osuga, T. S. Burcham and R. E. Feeney, *Anal. Biochem.*, 143 (1984) 62.
4 K. A. Schellenberg and J. Shaeffer, *Biochemistry*, 25 (1986) 1479.
5 M. Saha, G. M. Kresbach, R. W. Giese, R. S. Annan and P. Vouros, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 958.
6 R. B. Everson, E. Randerath, R. M. Santella, R. C. Cefalo, T. A. Avitts and K. Randerath, *Science (Washington, D.C.)*, 231 (1980) 54.
7 E. Randerath, T. A. Avitts, M. V. Reddy, R. H. Miller, R. B. Everson and K. Randerath, *Cancer Res.*, 46 (1986) 5869.
8 K. Vahankangas, A. Haugen and C. C. Harris, *Carcinogenesis (London)*, 6 (1985) 1109.
9 N. Koga, P. B. Inskeep, T. M. Harris and F. P. Guengerich, *Biochemistry*, 25 (1986) 2192.
10 K. Savela, K. Hemminki, A. Hewer, D. H. Phillips, K. L. Putman and K. Randerath, *Mutat. Res.*, 224 (1989) 485.
11 J. Bakthavachalam, R. S. Annan, F. A. Beland, P. Vouros and R. W. Giese, *J. Chromatogr.*, 500 (1990) 373.
12 F. Kadlubar, personal communication.