



Direct-acting mutagenicity of extracts of coal burning-derived particulates and contribution of nitropolycyclic aromatic hydrocarbons

Rina Taga^a, Ning Tang^a, Tetsuyuki Hattori^a, Kenji Tamura^b, Shigekatsu Sakai^c, Akira Toriba^a, Ryoichi Kizu^a, Kazuichi Hayakawa^{a,*}

^a Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

^b National Institute for Environmental Studies, 16-2, Onogawa, Tsukuba 305-8506, Japan

^c Hokkaido Institute of Environmental Science, Kita-19jo Nishi 12, Kita-ku, Sapporo 060-0819, Japan

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Abstract

Benzene–ethanol extracts from particulates produced by coal burning were separated into four fractions by silica-gel column chromatography using *n*-hexane (240 ml), *n*-hexane–dichloromethane (3:1, v/v) (200 ml), dichloromethane (200 ml) and methanol (450 ml), as the corresponding eluents. The mutagenicity of each fraction was assayed by the Ames test using the *Salmonella typhimurium* YG1024 strain. The nitropolycyclic aromatic hydrocarbons (NPAHs) of each fraction were assayed by high-performance liquid chromatography with chemiluminescence detection. The highest activity was observed in the *n*-hexane–dichloromethane fraction (Fr. 2). The mutagenic contribution of this fraction was 69.9% of the total of the four fractions. Ten of 11 NPAHs detected were in Fr. 2 and one (1-nitropyrene) was most concentrated in Fr. 3. Among the NPAHs examined, 3-nitrobenzanthrone made the largest mutagenic contribution. This is the first report of detection of 3-nitrobenzanthrone in coal burning-derived particulates.

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1. Introduction

Particulates released from the burning of coal contain compounds many kinds of organic pollutants such as carcinogens and/or mutagens. Inhalation of high concentrations of suspended particulate matter causes

* Corresponding author. Tel.: +81 76 234 4413;

fax: +81 76 234 4456.

E-mail address: hayakawa@p.kanazawa-u.ac.jp
(K. Hayakawa).

respiratory, cardiac and lung diseases. The particulates show direct- and indirect-acting mutagenicities, and nitropolycyclic aromatic hydrocarbons (NPAHs) are considered to be the main cause of the direct-acting mutagenicity, and polycyclic aromatic hydrocarbons (PAHs) to be the indirect-acting mutagenicity [1,2]. Several NPAHs such as, 1,3-, 1,6- and 1,8-dinitropyrenes (DNPs) and 3-nitrobenzanthrone (NBA) show very strong direct-acting mutagenicities [3,4]. These NPAHs are mainly formed through an imperfect combustion process. Some NPAHs are also known to be formed through secondary reactions of PAHs and nitrogen oxides in the atmosphere.

Diesel exhaust particulates (DEPs) cause serious urban air pollution in Japan [1,5,6]. We previously reported that the main contributors of direct-acting mutagenicity of DEPs were NPAHs including 1,3-, 1,6- and 1,8-DNPs, 1-nitropyrene (NP) [5,7] and 3-NBA [8]. In China and Russia, on the other hand, particulates exhausted from coal burning for industrial and domestic purposes have been considered as the main causes of recent urban air pollution [9–11]. Although several NPAHs were identified in coal fly ash [12], the contribution of NPAHs to direct-acting mutagenicity of coal burning-derived particulates (CBPs) is not known. In this study, to clarify the contribution of NPAHs to the mutagenic activity of CBPs, we measured the direct-acting mutagenicity of CBPs and the quantities of NPAHs in particulate extracts, collected from chimneys of coal-burning stoves.

2. Materials and methods

2.1. Chemicals

1,3-, 1,6- and 1,8-DNPs, 3-NBA, 2- and 9-nitroanthracenes (NAs) and 5-nitroacenaphthene (NAc) and 2-fluoro-7-nitrofluorene (FNF, internal standard) were obtained from Aldrich (Milwaukee, WI, USA). 1-NP and 2-nitrofluorene were purchased from Tokyo Kasei (Tokyo, Japan). 6-Nitrobenzo[*a*]pyrene (NBaP) and 3-nitroperylene (NPer) were purchased from Chemsyn Science Laboratories (Lenexa, KS, USA). 4- and 9-Nitrophenanthrenes (NPhs) and 7-nitrobenz[*a*]anthracene (NBaA) were obtained from Accu-standard Inc. (New Haven, CT, USA). 6-Nitrochrysene (NC) and 1-NPer were obtained from Chiron AS

(Trondheim, Norway). 3-Nitrofluoranthene (NFR) and 2-nitrotriphenylene (NTP) were obtained from Wako Pure Chemical Industries (Osaka, Japan) and Hayashi Pure Chemical (Osaka, Japan), respectively. 2- and 4-NPs were kindly provided by Dr. Y. Hisamatsu of the National Institute of Public Health (Tokyo, Japan). 10-NBA was kindly provided by Professor S. Fujisawa of the Faculty of Science, Toho University (Chiba, Japan). All other chemicals were special grade of commercially available products.

2.2. Sampling and pretreatment of CBP

Particulates were collected in Shenyang, China, from a chimney for a domestic coal stove by using a SL-15P low-volume air sampler (SIBATA, Tokyo, Japan) equipped with a T60A20 glass-fiber filter (SIBATA, Tokyo, Japan) at an air flow rate of 10 L/m³. The collected particulates (2.45 g) were extracted twice with benzene–ethanol (3:1, v/v) ultrasonically and the crude extract solution was obtained from filtering the solution through a cellulose fiber filter paper (Advantec, Tokyo, Japan).

2.3. Silica-gel column chromatography

After the crude extract solution was evaporated to dryness, the residue (7.5 mg) was redissolved in 10 mL of *n*-hexane and applied to a silica-gel column (Wakogel Q200, 1.5 i.d. × 21.3 cm bed). The column was then eluted successively with *n*-hexane (240 mL), *n*-hexane–dichloromethane (3:1, v/v) (200 mL), dichloromethane (200 mL), and methanol (450 mL), and the corresponding fractions (Frs. 1–4) were collected [13]. Each fraction was divided into two equal volume solutions and both solutions were evaporated to dryness. One dried sample was dissolved in 4 mL acetonitrile for the determination of NPAHs and PAHs, and the other was dissolved in 2 mL dimethylsulfoxide (DMSO) for the mutagenicity assay.

2.4. Determination of NPAHs

Twenty NPAHs were determined by our HPLC method [8,14]. The HPLC system consisted of five Shimadzu (Kyoto, Japan) LC-10A pumps, a Shimadzu SIL-10A auto sample injector, a Shimadzu DGU-14 degasser, a Shimadzu CLD-10A chemilu-

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