Development of determination method of nitropolycyclic aromatic hydrocarbons and study on atmospheric behaviors of polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons in East Asian cities

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学位授与の題目	Development of the Determination Method of Nitropolycyclic Aromatic Hydrocarbons and its Application for Study on Atmospheric Behaviors of Polycyclic Aromatic Hydrocarbons and Nitropolycyclic Aromatic Hydrocarbons in East Asian Cities（ニトロ多環芳香族炭化水素の分析法の開発と東アジアの都市における大気中多環芳香族炭化水素、ニトロ多環芳香族炭化水素の挙動研究への応用）
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学位論文要旨

ABSTRACT

Several polycyclic aromatic hydrocarbons (PAHs) and nitropolycyclic aromatic hydrocarbons (NPAHs), which mainly originate from imperfect combustion of organic matters such as coal and petroleum, are carcinogenic and/or mutagenic. We have found that several PAHs also have antiestrogenic and/or antiandrogenic activities. In recent years, consumption of energy sources including coal and petroleum has been increasing in developing countries in East Asia. Some researchers believe that pollutants from these countries will reach Japan by long-range transport, which means that they may have effects on eco-systems and human health. However, only a few studies have examined the emission and behavior of atmospheric PAHs and NPAHs in these countries. Therefore, it is necessary to analyze NPAHs, whose concentrations in the atmosphere are significantly lower than those of PAHs, sensitively and quickly, and to clarify the behavior of PAHs and NPAHs in these countries.

An automatic HPLC method with chemiluminescence detection (on-line method) for analyzing 1,3-, 1,6-, 1,8-dinitropyrene (DNPs) which are the strongest direct-acting mutagens and 1-nitropyrene (1-NP) in airborne particulates by adding on-line clean-up, reducer (Pt/Rh-coated alumina), whose maximum efficiency was achieved, and concentrator columns was developed. The on-line method was improved by introducing an ODS column to remove an interfering peak whose retention time was close to that of 1,6-DNP, and by increasing the loading time of the sample solution from 20 to 38 min and

increasing the flow rate of an ascorbic acid solution from 1.3 to 1.8 mL/min to analyze several additional compounds. Furthermore, a method based on the improved method for analyzing 1-, 2-, 3- and 10-nitrobenzanthrone isomers (NBA isomers) was developed. Using the pretreatment and reducing conditions of the improved method, 1-, 2-, 3- and 10-nitrobenzanthrone isomers (NBA isomers) were extracted from airborne particulates and reduced to their corresponding amino derivatives. Using 40% acetonitrile/60% 10 mM imidazole-$HClO_4$ buffer as a mobile phase at a flow rate of 1 mL/min, these NBA isomers were present in a fraction that eluted from a concentration column. They were well separated by a polymeric-type ODS column and detected by chemiluminescence detection.

Airborne particulates were collected in Shenyang (China), Vladivostok (Russia), Sapporo, Toyama, Kanazawa, Tokyo and Kitakyushu (Japan). The results comparing the concentrations and compositions of NPAHs in these cities suggested that the major contributor of atmospheric NPAHs was different between Shenyang, Vladivostok and Japanese cities. In spite of lighter traffic volume, the mean atmospheric concentrations of nine PAHs in Shenyang and Vladivostok were substantially higher than those in Tokyo, Sapporo, Kitakyushu and Kanazawa. However, the mean atmospheric concentrations of four NPAHs, which were directly exhausted from primary sources such as diesel-engine vehicles, were at the same levels in every city except for Kitakyushu. Furthermore, the ratios of concentration of six NPAHs to nine PAHs were significantly smaller in Shenyang and Vladivostok and the values were close to those of particulates in the exhaust from domestic coal-burning heaters. These results suggest that a large amount consumption of coal for power plants and domestic heaters in winter contributes to these pollutants in Shenyang and Vladivostok. On the other hand, indirectly formed NPAHs such as 2-NP and 2-NFR were also significantly higher in Shenyang and Vladivostok. The higher concentrations may be due to higher atmospheric concentrations of their mother PAHs. We also propose that the [NPAHs]/[PAHs] ratio is a useful indicator of not only diesel-engine vehicles (0.195) but also coal combustion (0.001).

INTRODUCTION

Several polycyclic aromatic hydrocarbons (PAHs) and nitropolycyclic aromatic hydrocarbons (NPAHs) are carcinogenic and/or mutagenic. The International Agency for Research on Cancer (IARC) has already ranked 1,6-, 1,8-dinitropyrene (DNPs), 1-, 4-nitropyrene (NPs) and 6-nitrochrysene (6-NC) to group 2B (agents that are possibly carcinogenic to humans). Furthermore, 3-nitrobenzanthrone (3-NBA) has been shown to be strongly mutagenic and/or carcinogenic. The mutagenicity of 3-NBA was comparable to that of 1,8-DNP which is the strongest mutagen by the Ames test using *Salmonella typhimurium* TA98 and YG1024 strains. In recent years, we have found that several PAHs had antiestrogenic and/or antiandrogenic activities.

PAHs and NPAHs need to be monitored because they are a health risk for humans

and are present throughout the environment. Howéver, less is known about atmospheric NPAHs than is known about atmospheric PAHs, because the concentrations of NPAHs are significantly lower than those of PAHs. Therefore, an ultrasensitive analytical method is necessary to determine atmospheric NPAHs. We previously developed an HPLC method with chemiluminescence detection (HPLC/CLD) for NPAHs. The detection limits were two orders of magnitude lower than those by HPLC/FLD or GC/MS. However, the HPLC/CLD method required tedious preparation procedures including cleaning up the NPAHs samples and chemically reducing them to the corresponding amino derivatives (APAHs).

In Section 1, to simplify the NPAHs analyses, we developed and improved an automatic HPLC method with chemiluminescence detection by adding on-line clean-up, reducer and concentrator columns.

On the other hand, the consumption of energy sources including coal and petroleum has been increasing in developing countries in East Asia in recent years. However, only a few studies have examined the emission and behavior of atmospheric PAHs and NPAHs in these countries. Therefore, in Section 2, the atmospheric behaviors of PAHs and NPAHs in East Asian cities were studied by comparing the analytical results of PAHs and NPAHs in airborne and coal stove exhaust particulates collected in Shenyang (China), Vladivostok (Russia), Tokyo, Toyama, Kanazawa, Sapporo and Kitakyushu (Japan).

RESULTS AND DISCUSSION

1. Development of Determination Method of NPAHs
   (1) An automatic HPLC system for analyzing NPAHs in airborne particulates was developed by adding on-line clean-up, reducer (Pt/Rh-coated alumina) and concentrator columns. This system reduced the analysis time by about 2 h, which was previously required for the clean-up and reduction processes. Trace levels of DNPs and 1-NP in airborne particulates were sensitively detected.
   (2) An interfering peak originating from an ascorbic acid solution that elutes at a retention time close to that of 1,6-DNP in the above system was effectively removed by introducing an ODS column just after the pump for the ascorbic acid solution. Besides three DNPs and 1-NP, 2-, 4-NPs, 2-nitrofluorene, 6-NC, 7-nitrobenz[*a*]anthracene, 3-nitropelyrene and 6-nitrobenzo[*a*]pyrene were also detected by increasing the loading time of the sample solution from 20 to 38 min, and by increasing the flow rate of the ascorbic acid solution from 1.3 to 1.8 mL/min using our improved system (Fig. 1).

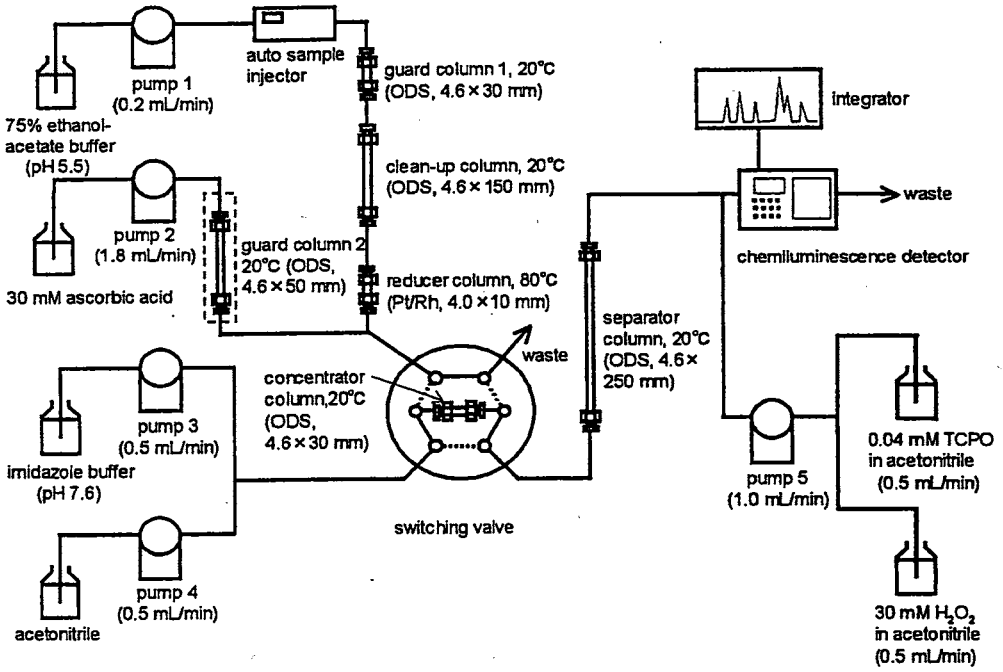

Fig. 1. Schematic diagram of the proposed NPAH analysis system.

(3) Under the pretreatment and reducing conditions as described above, 1-, 2-, 3- and 10-NBA isomers also could be extracted from airborne particulates and reduced to their corresponding amino derivatives. A mixture of 40% acetonitrile and 60% 10 mM imidazole-$HClO_4$ buffer as a mobile phase at a flow rate of 1 mL/min eluted these NBA isomers. They were then separated well by a polymeric-type ODS column (Cosmosil 5C-18MS, 4.6 i.d. X 250 mm) and detected chemilumigenically.

2. Atmospheric Behaviors of PAHs and NPAHs in East Asian Cities

(1) Airborne particulates were collected simultaneously in Vladivostok, Toyama and Kanazawa. Figure 2 shows the sampling cities in East Asia. Five unknown peaks which might be NPAHs were detected only in chromatograms of Vladivostok. This result suggested that the composition of atmospheric NPAHs in Vladivostok was different from that in Toyama and Kanazawa.

(2) The difference in traffic volume at two sites in Vladivostok did not have a significant effect on the concentrations of DNPs and NPs in contrast to two sites in Kanazawa. This result suggested other major sources than diesel-engine vehicles also contribute to DNPs and NPs in Vladivostok.

(3) The concentration differences between V-1 (light traffic site) and

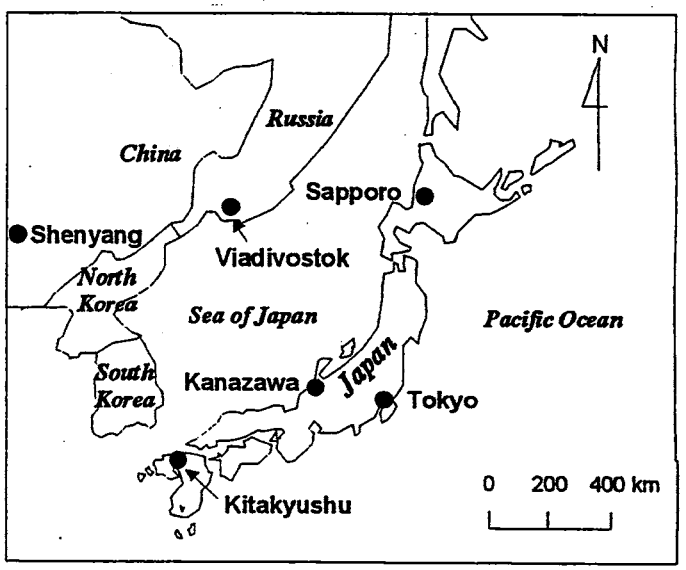

Fig. 2. Sampling cities in East Asia.

V-2 (heavy traffic site) in Vladivostok were significantly large in summer but not in winter. As a cause of high concentrations of NPAHs at V-1 in winter, other contributors such as city and domestic heaters should considered.

(4) As in Kanazawa, the concentration ratio of 2-NP to 1-NP ([2-NP]/[1-NP]) in Vladivostok was larger at V-1 than that at V-2. This suggested that, as in Kanazawa, 2-NP was formed in the atmosphere in Vladivostok.

(5) The mean atmospheric concentrations of PAHs in Shenyang and Vladivostok were substantially higher, in spite of lighter traffic volume than those in Tokyo, Sapporo, Kitakyushu and Kanazawa. However, the mean atmospheric concentrations of NPAHs were at the same levels in every city except for Kitakyushu. As a possible cause, the emission of a large amount of particulates containing high concentrations of PAHs but not high concentrations of NPAHs in Shenyang and Vladivostok was considered.

(6) Indirect formations of NPAHs such as 2-NP and 2-nitrofluoranthene were significantly higher in Shenyang and Vladivostok. The higher concentrations may be due to higher atmospheric concentrations of their mother PAHs.

(7) The [NPAHs]/[PAHs] ratios were significantly smaller in Shenyang and Vladivostok and the values were close to those of exhaust particulates from domestic coal-burning heaters. This result suggests that a large amount consumption of coal for power plants and domestic heaters, etc. in winter contributes to these pollutants in Shenyang and Vladivostok.

(8) The [NPAHs]/[PAHs] ratio of particulates emitted from coal stoves was significantly smaller than that of particulates emitted from diesel-engine vehicles. As a possible cause, the degree of nitration may be lower because the combustion temperature and amount of oxygen in coal stoves are lower than those in diesel engines. Therefore, the [NPAHs]/[PAHs] ratio is a useful indicator of not only diesel-engine vehicles but also coal combustion.

学位論文審査結果の要旨

[審査経過] 平成15年11月25日の第1回審査委員会で審査方針を決定した。基礎学力を確認し、各委員による面接と諮問を行い、12月15日に口頭発表（最終試験）を行った。終了後開催した最終審査委員会において協議の結果、次の結論を得た。

[審査結果] 今日の大気汚染は、ぜん息や肺がんの増加を招き深刻化している。その主な原因物質として、化石燃料の不完全燃焼で発生する粉じん（煤）とこれに付着する多環芳香族炭化水素（PAH）及びニトロ多環芳香族炭化水素（NPAH）が指摘されている。本研究は、まず、従来のNPAH分析システムを改良して、その分析対象化合物数を飛躍的に増加させるとともに、前処理の自動化も達成した。次いで、本システムを駆使して、日本（金沢、札幌、東京、北九州）、中国（瀋陽）、ロシア（ウラジオストク）及び韓国（ソウル）の都市大気汚染の解析を行い、次のことを明らかにした。即ち、中国及びロシアの都市大気中NPAH濃度は日本や韓国の都市と大差ないが、PAH濃度は著しく高い。この違いが、日本や韓国の主要発生源がディーゼル車であるのに対して、中国やロシアの主要排出源は、石炭を使用する家庭や工場から排出する多量のPAHを含む煙であることに起因することを明らかにした。本研究は、東アジアの大気汚染の実態解明のみならず、その施策の上でも重要な知見を提供しており、審査委員会は博士（薬学）に値すると判定した。