Identification of Chlorinated Polycyclic Aromatic Hydrocarbons

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are discharged from various emission source in air environment and in aqueous environment[1]. PAHs discharged in the atmosphere are flowed into aqueous environment through falling or rain water[2,3]. It seems PAHs are receiving the changes in the environment by chemical reaction, photoreaction, metabolism of the organism and various treatment processes. Especially, in the case of raw water for supply, these compounds are exposed chlorine in the process for the disinfection and then are reacted oxidation and/or chlorine displacement. It is known that compounds in water are changed to various reaction products by receiving these reactions. For instance, trihalomethanes and halo-acetic acids are occurred in the disinfection process as disinfection by products[4]. Among these compounds, there are many compounds causing the adverse effect to the human[5]. It is guessed that PAHs are also similarly changed to various reaction products in the process for the disinfection by the reaction of oxidation and/or chlorine displacement. Additionally, it is afraid the reaction products effect on human health[6,7]. There are few reports about behavior of PAHs during chlorine treatment in water treatment process. In this paper, we investigated the behavior of benzo[a]pyrene (B[a]P) and fluorenthene (FL) in the presence of chlorine by liquid chromatography electrospray mass spectrometry[8,9].

Materials and Methods

Chemicals

Benzo[a]pyrene (93.0%) and fluoranthene (97.0%) were purchased from Wako Pure Chemicals Industries, Ltd (Osaka, Japan). Acetonitrile and trifluoroacetic acid were purchased analytical grade from Wako Osaka, Japan. Pure water was prepared by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Benzo[a]pyrene and fluoranthene were dissolved in acetonitrile.

Liquid Chromatography Electrospray Mass Spectrometry (LC-ESI-MS) Analysis

LC was carried out an Agilent 1100 series (Agilent, Waldbornn, Germany) instrument equipped with a Rheodyne Model 7750 injector. The analytical column was a Zorbax Eclipse XDB-C_{18} (Agilent) 250mm x 4.6mm, 5um particle size. The mobile phases were 0.05% (v/v) trifluoroacetic acid (A) and 100% acetnitorile (B). Compounds were separated with the following gradient program: maintaing 80%B for 17min; following by a linear gradient from 80%A at *t*=17min to 100%B at *t*=17.5min; maintaining 100%B for 52.5min. The column temperature was 40^{o}C, the flow-rate was 1.0ml/min and the injection volume was 25ul. The MS system was an Agilent 1100series (Agilent) quadrupole equipped with an electrospray ionization (ESI) source. The instrument was operated in the positive ionization mode. The operating conditions for ESI were nebulizer gas (nitrogen) 414Kpa; drying gas (nitrogen) flow 10liter/min; capillary voltage 4000V and gas temperature 350^{o}C. The fragmentor voltage was kept at 120V.

Condition of Chlorination

The pure water was fortified with 1ml of 0.1M phosphate buffer (pH 7.0) and was made the concentration of free residual chlorine ion to be 1ppm by adding sodium hypochlorite. Then, the solution of Benzo[a]pyrene or fluoranthene was dropped into the chlorinated water to be 3.0ug/l final concentration. Benzo[a]pyrene or fluoranthene was made to contact the chlorine ion at 20^{o}C, while the water sample was stirred with stirrer.

Analytical procedure

L (+)-ascorbic acid sodium salt (Wako, Japan) was added to the sample water to 0.005% (w/v). The sample water adjusted to pH3.5 by 9% (v/v) nitric acid and then concentrated with solid phase extraction (SPE) method using Oasis HLB Plus Extraction Cartridges (Waters; Milford, MA, USA) prepacked with *N*-vinyl-pyrrolidone polymer resin as described previously[10]. 1 liter of each sample was extracted with the SPE cartridges equilibrated with 5ml dichloromethane, 5ml methanol and the 5ml pure water. Extraction of water samples was carried out at a 10ml/min flow rate. After passing the sample through the cartridges, cartridges were washed with 10ml pure water at a 5ml/min. Air was then pulled through the cartridge for 40min. The analytes were eluted from the cartridges with 3ml acetonitrile at a rate of 1-2 drops/sec. After evaporating the samples to near dryness under a gentle nitrogen stream, the compounds were transferred into a final volume of 1.0ml of acetonitrile[9,10].

Results and Discussion

Chlorination of Benzo[a]pyrene and Fluoranthene

The pure water was fortified with 1ml of 0.1M phosphate buffer (pH 7.0) and made the concentration of free residual chlorine ion to be 1mg/l by adding sodium pypochlorine. This concentration of free chlorine is the water quality standard value of tap water in Japan. If PAHs are remained in the tap water, PAHs might react with chlorine at this concentration or less. The solutions of Benzo[a]pyrene (B[a]P) or Fluoranthene (FL) were dropped into the chlorinated water to be 3.0mg/l final concentration and the water samples were kept to stir at 20^{o}C.

Benzo[a]pyrene

Although the concentration of the free chlorine was decreased slightly on the depend of contact time, the free chlorine was remained over 80% (more than 0.8ppm) of the initial concentration after 48 hours contact. This means the necessary quantity of chlorine for the reaction had sufficiently remained after 48hours and that the reaction had almost perfectly advanced. The LC/MS system was used for quantitative analysis of chlorinated B[a]Ps and chlorinated FLs[8,9]. The use of high-flow pneumatically assisted ESI run in positive mode in a soft ionization technique. In this study, B[a]P (m/z;252 and 253), mono-chlorinated B[a]P (m/z;286 and 288) and di-chlorinated B[a]P (m/z;320 and 322) could be detected in the selected ion monitoring. The LC/MS chromatograms for detection of B[a]P, mono-chlorinated B[a]P and di-chlorinated B[a]P are shown in Fig.1. After contact of chlorine, B[a]P rapidly decreased and then reached to 6.6% of the initial concentration after 2 hour contact (Fig.1). The peak area of mono-chlorinated B[a]P (*t*=25.9min) increased depending on the contact time with chlorine, as B[a]P decreased. The concentration of mono-chlorinated B[a]P kept constantly from 24 hours and 48 hours. The peak area of di-chlorinated B[a]P was detected at 2hours and increased until 4 hours. Di-chlorinated B[a]P was changed from 4 hours and 48hours at the constant concentration. From these results B[a]P rapidly is reacted with the chlorine and is changed to the chlorinated forms. First, B[a]P is changed to mono-chlorinated form and gradually changed to di-chlorinated form. In addition, it seems di-chlorinated form is substituted more with chlorine or is decomposed.

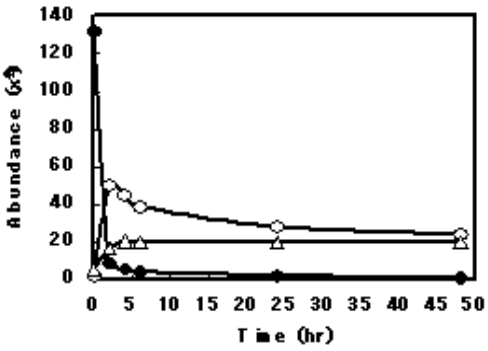

Fig.1 Fluctuations of B[a]P and Its Chrolinated forms

Fluoranthene

Although the concentration of the free chlorine was decreased slightly on the depend of contact time, the free chlorine was remained over 84 % (more than 0.84ppm) of the initial concentration after 24 hours (data not shown) and the necessary quantity of chlorine for the reaction had sufficiently remained in this case during examination. In this study, FL (m/z;202 and 101), mono-chlorinated FL (m/z;236 and 135) could be detected in the selected ion monitoring (SIM). After contact chlorine, FL rapidly

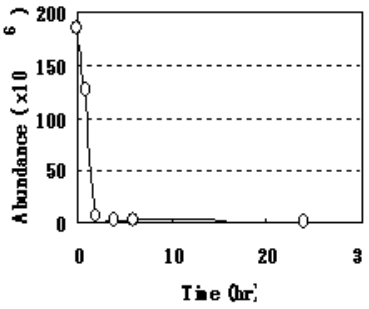

Fig.2 Fluctuation of Fluoranthene

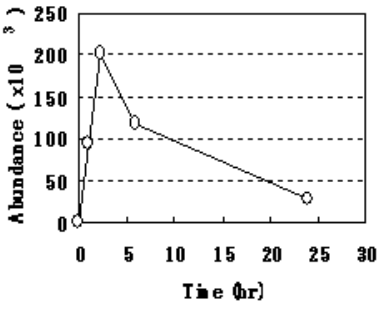

Fig.3 Fluctuation of Chlorinated Fluoranthene

decreased and reached to 3.2% of the initial concentration after 2 hour contact (Fig.2). The peak area of mono-chlorinated FL (*t*=17.5min) increased depending on the contact time with chlorine until 2 hours. However, mono-chlorinated FL decreased and reached about 12% of highest value (at 2 hours). Di-chlorinated and trichlorinated FL could not be detected until 24hours. From these results, FL israpidly reacted with the chlorine and changed to mono-chlorinated form, but mono-chlorinated form is not stable in comparison with chlorinated B[a]Ps. It seems mono-chlorinated FL or FL itself is decomposed with chlorine.

In this paper, we show PAHs might react with chlorine and change to chlorinated form. It is considered that the chlorination mechanism of PAHs is individually different. It is important to advance the elucidation of the mechanism of the chlorination reaction of PAHs and the effect evaluation to the organism.

Acknowledgements

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References

1. Mabilia R., Cecinato A., TomasiSciano M.C., Di Palo V. and Possanzini M. (2004) Characterization of polycyclic aromatic hydrocarbons and carbonyl compounds in diesel exhaust emissions. *Ann. Chim.* 94, 733-740.

2. Murakami M., Nakajima F. and Furumai H. (2004) Modelling of runoff behaviour of particle-bound polycyclic aromatic hydrocarbons (PAHs) from roads and roofs. *Water Res*. 38, 4475-4483.

3. Kamalakkannan R., Zettel V., Goubatchev A., Stead-Dexter K. and Ward N. I. (2004) Chemical (polycyclic aromatic hydrocarbon and heavy metal) levels in contaminated strmwater and sediments from a motorway dry detection pond drainage system. *J. Environ. Muonit.* 6, 175-181.

4. Toledano M. B., Nieuwenhuijsen M. J., Best N., Whitaker H., Hambly P., de Hoogh C., Fawell J., Jarup L. and Elliott P. (2005) Relation of trihalomethane concentrations in public water supplies to stillbirth and birth weight in three water regions in England. *Environ. Health Prespect*. 113, 225-232.

5. St-Pierre A., Krishnan K. and Tardif R. (2005) Chearacterization of the metabolic interaction between trihalomethanes and chloroacetic acids using rat liver microsomes. *J. Toxicol. Environ. Health A*, 68, 287-298.

6. Bhatia A. L., Tausch H., and Stehlik G. (1987) Mutagenicity of chlorinated polycyclic aromatic compounds. *Ecotoxicol. Environ. Saf.* 14, 48-55.

7. Oyler A. R., Liukkonen R. J., Lukasewycz M. K., Cox D. A., Peake D. A. and Carlson R. M. (1982) Implications of treating water containing polynuclear aromatic hydrocarbons with chlorine: a gas chromatographic-mass spectrometric study. *Environ Health Prespect.* 46, 73-86.

8. Ayano E., Kanazawa H., Ando M. and Nishimura T., (2004) Determination and quantitation of ulfonylurea and urea herbicides in water samples using liquid chromatography with an electrospray mass detector. *Anal. Ckim. Acta,* 507,211-218.

9. Ayano E., Okada Y., Sakamoto C., Kanazawa H., Okano T., Ando M. and Nishimura T. (2005) Analysis of

herbicides in water using temperature-responsive chromatography and an aqueous mobile phase. , *J.Chromatogr. A* 1069, 281-285.

10. Jeannot R., Sabik H., Sauvard E. and Genin E. (2000) *J. Chromatogr*. A 879:51-71.