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Relationship of particulate matter and ozone with 3-nitrotyrosine in the atmosphere☆

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ABSTRACT

The prevalence of allergic diseases has increased in the past few decades. Bio-aerosol proteins and their chemical modifications, such as 3-nitrotyrosine (3-NT), in the atmosphere have been attracting attention due to their promotive effects on allergies. 3-NT is generated from the amino acid, tyrosine, through a reaction with ozone (O_3) and nitrogen dioxide (NO_2). However, the underlying mechanisms have not yet been elucidated in detail. Therefore, we measured 3-NT and evaluated the relationships among 3-NT and various pollutants such as sulfur dioxide (SO_2), NOx ($NO + NO_2$), ozone (O_3), PM_7, total suspended particulate matter (TSP) containing proteins, humidity, and temperature. 3-NT positively correlated with O_3, SO_2, humidity, and temperature, and negatively correlated with NOx. A multiple regression analysis showed that 3-NT positively associated with O_3, humidity, and PM_7. O_3 positively associated with 3-NT and PM_7, and negatively associated with NOx and humidity. These results suggest that 3-NT is generated from PM proteins through a reaction with O_3 under high humidity conditions, and that the measurement of 3-NT is important and useful for the research of O_3.

1. Introduction

Ozone (O_3) is a well-known atmospheric air pollutant, a so-called “photochemical oxidant”. Although the mean concentration of O_3 in Japan has generally remained unchanged in recent years, it has increased in urban areas with large populations in the past two years. The concentration of O_3 in many urban areas in the U.S., including New York City, has been shown to exceed the National Ambient Air Quality Standard (NAAQS) (Sheffield et al., 2015). The adverse effects of O_3 on the respiratory system have been established using animal experiments and in epidemiological studies (Sheffield et al., 2015).

Various components of biological aerosol particles in atmospheric particulate matter (PM) include viruses, pollen, fungal spores, bacteria, and debris from vertebrates, such as humans, and other biota (including plants and insects) (Castillo et al., 2012). Various proteins have been identified in bio-aerosol (Jaenicke, 2005; Castillo et al., 2012). However, some bio-aerosol proteins may form complexes with airborne PM. Protein, accounting for up to ~5% of urban PM, exerts physicochemical effects on atmospheric particles, and plays major roles as airborne allergens in wind-driven and traffic-related suspensions of soil and road dust (Franze et al., 2005). A previous study reported that the protein content in the urban aerosol ranged between 0.5% and 2% throughout the year in all size fractions (Abe et al., 2016). Proteins in PM are chemically modified by various pollutants such as O_3, nitrogen dioxide (NO_2), and sulfur dioxide (SO_2) (D'Amato et al., 2007).

Bet v 1, an allergic protein of birch pollen, has been detected in PM, and tyrosine-nitrated Bet v1 exhibits enhanced allergenic potential (Franze et al., 2005). O_3 and NO_2 have been shown to promote the nitration of protein molecules, with the formation of 3-NT being reported in polluted urban air (Bolzacchini et al., 2001; Shiraiwa et al., 2012). 3-NT has been detected in dust samples from various urban environments. The addition of nitro-groups on the aromatic rings of tyrosine residues in the polypeptide chain enhances the allergenic potential of proteins (Gruijthuijsen et al., 2006). However, these findings were obtained in an experiment using a pseudo-atmospheric space. Since it is possible to measure 3-NT in the back filters of a high-volume air sampler with a high

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sensitivity technique using a high performance liquid chromatography-electrochemical detector (HPLC-ECD), we evaluated the relationships among 3-NT and ozone, NO, PM proteins, and other pollutants in samples collected with an air sampler and Government data.

2. Materials and methods

2.1. Source of air PM

Airborne PM in total suspended particulates (TSP) was collected with a high-volume air sampler (HV-1000F, SHIBATA, Souka City, Japan) using a quartz filter (8 inches × 10 inches, 2500QAT-UP, Pallflux Products, Putnam, CT) at a flow rate of 1000 L/min continuously for 4 or 7 days between February 2014 and February 2015 in Okayama city, Okayama Prefecture, Japan. Filters were stored in a freezer (−30 °C).

2.2. Air pollutants and meteorological elements

Data on the 1-h concentrations of SO_2, NOx, O_3, and PM_7 were obtained from the Okayama Prefecture Institute for Environmental Science and Public Health, and PM_7 was simultaneously collected for airborne PM. PM_7 is defined as the amount of PM with an aerodynamic diameter less than 10 μm obtained using an impactor with a 100% cut-off point, as stated by the Japanese Air Quality Standard. The 50% cut-off diameter of PM_7 is assumed to be approximately 7 μm; therefore, we hereafter referred to this variable as PM_7. The monitoring station at which these air pollutants were measured was located on a residual street in the city. Meteorological elements of 1-hr mean values for relative humidity and temperature were obtained from the Japan Meteorological Agency.

2.3. Measurement of TSP proteins and 3-NT

TSP protein concentrations were measured using the bicinchoninic acid (BCA) method (Thermo Fisher Scientific, Rockford, IL, USA). A round hole with a diameter of 6 mm was cut into a TSP quartz filter, which was then placed in 500 μl of reagents of BCA and warmed at 37 °C for 30 min. After the reaction, samples were centrifuged at 5000 rpm for 10 min, and the protein concentration in the supernatant was measured spectrophotometrically at 562 nm.

Regarding the measurement of 3-NT, a pore with a diameter of 6 mm was cut into a TSP filter collected from a high volume air sampler, and contaminated proteins in this TSP filter were then reacted with pronase (Roche, Mannheim, Germany) to hydrolyze proteins to amino acids in 300 μl of 0.1 M citrate buffer at 60 °C for 16 h. After the reaction, vials were centrifuged at 5000 rpm for 10 min, and the supernatant was filtered with 10-kDa Amicon. A filtered sample (25 μl) was applied to HPLC-ECD (Hitomi et al., 2007). The concentrations of TSP proteins and 3-NT in the atmosphere were calculated from the total amounts of protein and 3-NT in the quartz fiber filter and filtered air during the collection time, and the units of TSP proteins and 3-NT were expressed as $\mu g/m^3$ and pg/m^3, respectively.

2.4. Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.0c for Mac (GraphPad Software, Inc., San Diego, CA) and PASW Statistics 18 for Mac (SPSS Inc., South Wacker Drive, Chicago). Results are expressed as the mean ± standard of error (SE). Spearman's correlation was used for the correlation analysis. The mean values of O_3, NOx, SO_2, PM_7, humidity, temperature, and TSP protein concentrations were evaluated according to the quartiles of 3-NT by a one-way ANOVA and trend analysis. The relationships among 3-NT or O_3 and other pollutants and meteorological elements were analyzed by a multiple regression analysis using transformed log10 format variables such as 3-NT, SO_2, NOx, PM_7, temperature, and untransformed variables including O_3 and humidity. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. Characteristics of atmospheric pollutants and meteorological elements

The characteristics of atmospheric pollutants such as 3-NT, O_3, NOx ($NO + NO_2$), SO_2, PM_7, humidity, temperature, and TSP proteins were shown as means ± SE (Table 1).

3.2. Relationships among 3-NT or TSP proteins and pollutants and meteorological elements

The correlations among 3-NT or TSP proteins and pollutants and meteorological elements were shown in a correlation matrix (Table 2). 3-NT positively correlated with SO_2, O_3 (Fig. 1A), PM_7, humidity (Fig. 1C) and temperature, and negatively correlated with NOx. TSP proteins correlated with NOx and PM_7. O_3 positively correlated with 3-NT, SO_2, PM_7, and temperature, and negatively correlated with humidity (Fig. 1B).

When 3-NT was divided into quartiles, NOx decreased, while SO_2, PM_7, and temperature increased with elevations in 3-NT (Table 3).

3.3. Multiple regression analysis for 3-NT and ozone

The multiple regression analysis for 3-NT and ozone by the stepwise method was shown in Table 4. 3-NT positively associated with PM_7, O_3, and humidity. O_3 negatively associated with NOx and humidity, and positively associated with 3-NT and PM_7.

3.4. Seasonal changes in 3-NT

3-NT concentrations were elevated in May, June, July, August, and September (Fig. 2), but were low between November and February, with the lowest concentrations being recorded in December. Maximum UV levels were noted between May and September (Fig. 2).

4. Discussion

The present results demonstrated the positive association of 3-NT with O_3, humidity, and PM in the atmosphere using a regression analysis. Regarding the generation of 3-NT in the atmosphere, the involvement of NO_3 radicals and O_3 was demonstrated in

Table 1
Characteristics of atmospheric pollutants.

Atmospheric pollutants	N	Mean	± SE
3-Nitrotyrosine pg/m^3	56	22.07	±3.54
O_3 ppb	56	27.11	±1.12
NOx ppb	56	13.16	±0.59
SO_2 ppb	56	4.77	±0.17
PM_7 $\mu g/m^3$	56	22.24	±1.44
Humidity %	56	66.60	±1.02
Temperature °C	56	16.93	±117
TSP protein $\mu g/m^3$	56	4.33	±0.22

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