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Biochemical and cellular effects of electrophiles present in ambient air samples

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ABSTRACT

Ambient vapor-phase samples collected in Riverside, California had shown that both redox and electrophilic activity were present, with the vapor phase containing higher levels of electrophiles than the particle phase. In this study, the biochemical effects of the vapor-phase electrophiles were examined using the purified thiol proteins, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), protein tyrosine phosphatase 1B (PTP1B) and KELCH-1 like ECH-associated protein 1 (Keap1). The results demonstrated that the thiol proteins were inactivated by the vapor-phase samples through covalent modifications. Next, two cellular responses, epidermal growth factor receptor (EGFR)/mitogen-activated protein (MAP) kinase and NF-E2-related factor 2 (Nrf2), to the ambient vapor-phase samples were assessed in A549 and RAW 264.7 cell lines, respectively. The vapor-phase samples, at non-oxidative concentrations, increased phosphorylation of EGFR, which is negatively regulated by PTP1B, and its downstream MAP kinase, extracellular signal-regulated kinase (ERK)1/2. Activation of Nrf2, which requires Keap1 alkylation, and expression of its downstream proteins were also observed. The electrophilic compounds present in ambient vapor-phase were shown to modify cellular proteins through covalent modification and to activate diverse cellular responses that can lead to inflammatory and adaptive responses.

1. Introduction

Numerous studies (Frampton et al., 1999; Verones and Oortgiesen, 2001; Veronesi et al., 2002; Calcabrini et al., 2004; Donaldson et al., 1997; Billet et al., 2007; Rumelhard et al., 2007) have described the toxicological properties of airborne particulate matter (PM), but little attention has been paid to the volatile components of ambient air that could initiate toxicological changes upon exposure. The participation of vapor-phase components of ambient air in the toxicity of air samples has been demonstrated in the studies using freshly emitted gasoline and diesel engine exhaust (Lund et al., 2007; Seagrave et al., 2003). The semi-volatile organic compounds were found to be more toxic than those in the particles.

This laboratory has been studying the chemical properties of PM as they relate to two common mechanisms of toxicity, the ability to generate reactive oxygen species (ROS) (Cho et al., 2005) and the ability to form covalent bonds (Shinyashiki et al., 2008, 2009). As part of an extended study of air samples from sites in the Los Angeles Basin (LAB), we have initially conducted collection campaigns in Riverside, California, a so called receptor site in which an air mass generated in the west side of the LAB is modified by components in the atmosphere and by light to undergo chemical changes (Eiguren-Fernandez et al., in press). In these collections, we were interested in collecting particles and vapors simultaneously. The results of the chemical analyses showed electrophiles to be present in both the particle and vapor-phase, but the electrophilic activity in the vapor-phase was 15-fold higher than that in the particle phase (Eiguren-Fernandez et al., in press). As electrophiles form covalent bonds with cellular nucleophiles, their actions could be long lasting causing different responses from those resulting from increases in cellular ROS, which can be reduced by cellular antioxidant mechanisms.

In prior studies, the electrophilic quinone, 1,2-naphthoquinone (1,2-NQ), and protein tyrosine phosphatase 1B (PTP1B) were found to undergo a Michael addition reaction resulting in covalent bond formation (Iwamoto et al., 2007). PTP1B is a negative regulator of the epidermal growth factor receptor (EGFR) (McCole et al., 2007), a receptor tyrosine kinase associated with multiple cellular responses including apoptosis, inflammation and cell proliferation

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(Jorissen et al., 2003), and 1,2-NQ was shown to increase levels of phosphorylated EGFR and proteins of the mitogen-activated protein (MAP) kinase pathway as a result of PTP inhibition (Iwamoto et al., 2007). In a recent study we showed that 1,2-NQ also activated the transcription factor, NF-E2-related factor 2 (Nrf2), in zebra fish by interacting with the cysteine groups on KELCH-1 like ECH-associated protein 1 (Keap1) (Kobayashi et al., 2009), the negative regulator of Nrf2. Nrf2 is associated with the expression of multiple protective proteins including γ-glutamylcysteine ligase (GCL), NADPH: quinone reductases and phase II biotransformation enzymes (Kobayashi et al., 2006). Inactivation of Keap1 either by thiol oxidation or alkylation causes the release of Nrf2 from an inactive complex, resulting in its migration to the nucleus where it interacts with the antioxidant/electrophile response element (ARE/EpRE) to initiate transcription (Satoh et al., 2006).

The high levels of electrophiles in the ambient vapor-phase led us to focus initially on the biochemical and cellular effects of this phase, and this manuscript describes results of those studies. Thus, samples of organic extracts of XAD-4 resin traps of the vapor-phase were examined for their ability to interact with three thiol proteins and the downstream cellular responses to the actions on two, which are related to the MAP kinase pathway and antioxidant responses.

2. Methods

2.1. Vapor-phase sample collection and extraction

Sample collection and extraction were conducted as reported elsewhere (Eiguren-Fernandez et al., in press). Vapor-phase samples were collected at Riverside, California using 20 g of XAD-4 resin (Acros, Thermo Frisher Scientific). This study utilized three samples collected during April and May in 2007 (RIV041607, RIV042507 and RIV050707) with ~400 m^3 of air volume per sample. The XAD-4 resin was extracted with dichloromethane, and solvent exchanged to DMSO with the final concentration of ~250 m^3 mL^{-1}. The XAD-4 extracts of RIV041607, RIV042507 and RIV050707 are referred to herein as VP1, VP2 and VP3, respectively.

2.2. Enzyme inactivation by vapor-phase samples

Chicken and rabbit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Sigma Chemical Company (MO). Recombinant human PTP1B and mouse Keap1 were prepared as described previously (Iwamoto et al., 2007; Kobayashi et al., 2006). A two-stage incubation method was employed to assess the inactivation of GAPDH and PTP1B by air samples as described previously (Shinyashiki et al., 2008; Iwamoto et al., 2007). Experimental details are described in each figure legend.

2.3. Biotin-$PEAC_5$-maleimide (BMI)-labeling assay

A BMI-labeling assay was utilized to determine the availability of protein thiol residues after incubation with the vapor-phase samples (Fig. 1). In the procedure, the target protein was allowed to react with vapor-phase samples, followed by incubation with BMI (Dojindo, Kumamoto, Japan). A decrease in biotin binding reflects the amount of modified thiols. Thus, 1 μg of either rabbit GAPDH, PTP1B or Keap1 was incubated with vapor-phase samples (VP1, 2.43 m^3 mL^{-1}; VP2, 2.50 m^3 mL^{-1}; VP3, 1.77 m^3 mL^{-1}) in 10 μL of 20 mM Tris–HCl (pH 8.5) at 25 °C for 60 min. An aliquot of the incubation mixture was mixed with 15 μM BMI and incubated at 25 °C for 30 min. Vapor-phase samples did not affect the labeling efficiency of BMI under the condition. The protein samples were then subjected to SDS-PAGE and Western blotting. The protein

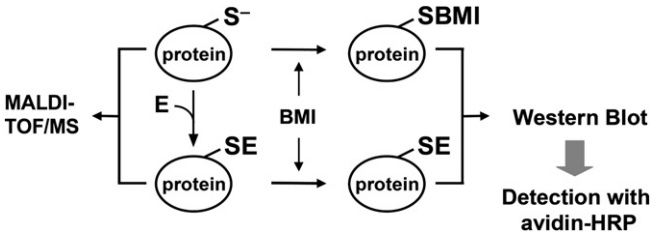

Fig. 1. BMI-labeling assay and MALDI-TOF MS can detect electrophiles binding to protein. E, electrophile. S^-, protein thiolate.

bound biotin was detected by horseradish peroxidase-conjugated streptavidin (HRP-avidin) using the enhanced chemiluminescence system. The membranes were also stained with coomassie brilliant blue (CBB) to confirm that the proteins were equally loaded.

2.4. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/TOF MS)

Two μg of rabbit GAPDH, PTP1B or Keap1 was incubated with DMSO or vapor-phase samples (VP1, 2.43 m^3 mL^{-1}; VP2, 2.50 m^3 mL^{-1}; VP3, 1.77 m^3 mL^{-1}) for 30 min at 25 °C in 20 mM Tris–HCl (pH 8.5). After mixing with a saturated solution of 3,5-dimethoxy-4-hydroxycinnamic acid containing 75% acetonitrile and 0.1% trifluoroacetic acid, the mixture was dried on stainless steel targets at room temperature. Positive ion MALDI-TOF mass spectra were acquired over the range m/z 25,000–90,000 using a Shimazdu AXIMA-TOF^2 mass spectrometer (Kyoto, Japan), which was operated in linear mode.

2.5. Cell culture

Human lung epithelial cell line, A549 (ATCC, Manassas, VA), and mouse macrophage cell line, RAW 264.7 (ATCC, Manassas, VA), were grown at 37 °C in a humidified atmosphere containing 5% CO_2 in Dulbecco's modified Eagle's medium supplemented with 10%fetal bovine serum, 2 mM GlutaMax-1 (Invitrogen, Carlsbad, CA) and antibiotics (100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin).

2.6. Intracellular production of oxidizing species

Production of oxidizing species in RAW 264.7 was monitored using 2′,7′-dichlorodihydrofluorescin diacetate (H_2DCFDA) (Invitrogen, Carlsbad, CA) by the method of Narayanan et al (Narayanan et al., 1997). Cells were pretreated with H_2DCFDA (10 μM) for 30 min prior to the incubation with vapor-phase samples for 10 min. Fluorescence of DCF was observed under fluorescence microscope (Leica, DMIRE-2, Germany). Hydrogen peroxide (H_2O_2) (10 mM) was used as a positive control.

2.7. Western blotting

Protein phosphorylation and expression were determined by Western blotting as previously described (Iwamoto et al., 2007).

2.8. Luciferase assay

The luciferase assay was conducted by the method previously described (Toyama et al., 2007) with modification. The ARE-luciferase reporter plasmid (a generous gift from Drs. Masayuki Yamamoto and Ken Itoh) consisted of glutathione S-transferase (GST) Ya promoter construct and luciferase gene. Transfection of ARE-luciferase plasmid into RAW 264.7 was performed by Polyfect (Qiagen, Valencia, CA) according to the manufacturer's instructions.