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Fractionating ambient humic-like substances (HULIS) for their reactive oxygen species activity – Assessing the importance of quinones and atmospheric aging

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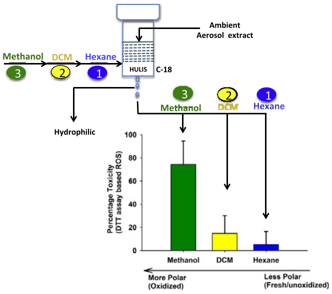
HIGHLIGHTS

- A novel technique to fractionate ambient humic-like substances (HULIS).
- HULIS subfractions linked with the reactive oxygen species (ROS) were identified.
- HULIS components associated with ROS are polar in nature.
- Oxidative aging of the atmospheric organic aerosols increases their ROS activity.

GRAPHICAL ABSTRACT



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ABSTRACT

In this paper, we present a technique to identify the redox-active components of fine organic aerosols by fractionating humic-like substances (HULIS). We applied this technique to a dithiothreitol (DTT) assay – a measure of the capability of PM to generate reactive oxygen species (ROS), and assessed the contribution of quinones to the DTT activity of ambient water-soluble PM. Filter samples from the Southeastern Center for Air Pollution & Epidemiology (SCAPE) were extracted in water and then passed-through a C-18 column to isolate the HULIS fraction by retention on the column. The HULIS was then eluted with a sequence of solvents of increasing polarity, i.e., hexane, dichloromethane (DCM) and then methanol. Each of these eluted fractions was analyzed for DTT activity. The methanol fraction was found to possess most of the DTT activity (>70%), while the hexane fraction had the least activity (<5%), suggesting that the ROS-active compounds of ambient water-soluble $PM_{2.5}$ HULIS are mostly polar in nature. A number of quinones thought to contribute to ambient PM DTT activity were also tested. 1,4 Naphthoquinone (1,4 NQ), 1,2 Naphthoquinone (1,2 NQ), 9,10 Phenanthrenequinone (PQ), and 5-hydroxy-1,4 NQ were analyzed by the same protocol. The hexane fraction of two quinones (PQ, and 1,4 NQ) was the most-DTT active, while methanol was the least, confirming that PQ, 1,4 NQ, and 1,2 NQ (which could not be recovered from the column) do not contribute significantly to the water-soluble DTT activity of ambient $PM_{2.5}$. However, an oxygenated derivative of 1,4 NQ, (5-hydroxy-1,4 NQ), which is also intrinsically more DTT-active than 1,4 NQ, was mostly (>60%) eluted in methanol. The results demonstrate the importance of atmospheric aging (oxidation) of organic aerosols in enhancing the ROS activity of ambient PM.

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1. Introduction

Fine particulate matter ($PM_{2.5}$) has been recognized as one of the most important air quality components affecting public health (Kappos et al., 2004; Lu et al., 2015; Oberdorster, 2001; Pope and Dockery, 2006; Pope et al., 2004). The mechanisms of action of the particulate matter (PM) causing health effects are not completely known, but one theory emerging in recent years is the burst of reactive oxygen species (ROS) following exposure of the biological system to PM (Akhtar et al., 2010; Donaldson et al., 2001; Gurgueira et al., 2002; King and Weber, 2013; Li et al., 2003a, 2009a; Venkatachari and Hopke, 2008). These ROS could be either carried by the particles themselves (King and Weber, 2013; Venkatachari and Hopke, 2008) or generated in-vivo (Delfino et al., 2013; Li et al., 2003b; Tao et al., 2003). The latter could involve several sequential mechanisms and has generally been linked with the PM chemical composition, such as fraction of redox-cycling organic compounds and metals (Biswas et al., 2009; Charrier and Anastasio, 2012; Verma et al., 2011, 2012, 2010).

Among the many methods developed to simulate ROS generation catalyzed by ambient PM (Ayres et al., 2008; Cho et al., 2005; DiStefano et al., 2009; Godri et al., 2011; Mudway et al., 2004; Shinyashiki et al., 2009) is the dithiothreitol (DTT) assay (Cho et al., 2005). In this assay, DTT acts as a donor of electrons, which are then transferred by certain PM components to oxygen leading to the generation of superoxide radical, which can go on to form stronger oxidants, such as the hydroxyl radical (Li et al., 2003a). Thus, the assay replicates the same mechanism as in mitochondria for ROS generation resulting from aerobic respiration. The DTT assay has been widely used to measure ROS generation capability of the ambient particles (conveniently called the ROS activity) collected from many locations in North America (Akhtar et al., 2010; Charrier and Anastasio, 2012; Cho et al., 2005; McWhinney et al., 2013a; Saffari et al., 2014; Verma et al., 2009a, 2009b, 2012). The time and labor-intensive protocol of the assay had generally limited the number of samples analyzed in the past studies (prior to 2014), however, recently a semi-automated instrument developed by Fang et al. (2014) facilitated the application of the assay to large sample sets, e.g. those collected from the southeastern United States during SCAPE (Southeastern Center for Air Pollution & Epidemiology) study [N ~ 500; (Verma et al., 2014)].

Various chemical components of the ambient PM have been linked to a response of the DTT assay. For example, quinones are one of the most important class of redox-cycling compounds that are known to oxidize DTT (Kumagai et al., 2002). In fact, 9,10-Phenanthrenequinone (PQ) has generally been used as a positive control in this assay by many research groups (Charrier and Anastasio, 2012; Fang et al., 2014; Li et al., 2009b). In addition, 1,2-Naphthoquinone (1,2 NQ) and 1,4-Naphthoquinone (1,4 NQ) have also been identified as DTT-active, but with lesser intrinsic activity than PQ (Li et al., 2009b). The strong reactivity of these quinones towards DTT has led some earlier researchers to conclude that most of the DTT activity of ambient PM is driven by these three quinones (Chung et al., 2006; Venkatachari and Hopke, 2008; Wang et al., 2011). However as shown by Charrier and Anastasio (2012), the atmospheric concentrations of quinones are generally very low (typically less than 2000 pg/m^3 of air) making it difficult to explain their dominant contribution to the DTT activity of ambient PM. Recent chamber studies, e.g. McWhinney et al. (2013b), also suggest that there are many more compounds in the redox-active organic aerosols, other than the small quinones generally measured in the ambient atmosphere, driving the DTT activity of ambient PM.

In contrast to these individual component approaches, other studies have focused on the bulk aerosol fractions associated with the DTT activity of ambient PM. Here researchers have used specific properties of the PM components such as volatility (Biswas et al., 2009; Verma et al., 2011), complexation affinity (Lin and Yu, 2011) and hydrophobicity (Verma et al., 2012) to remove the bulk fractions of organic compounds and/or metals and measured the effect of removal of each of these fractions on the residual ROS activity. For example, a class of water-soluble organic compounds segregated by their strong hydrophobicity, known as humic-like substances (HULIS) has been identified as a major group of species associated with ROS generation (Verma et al., 2015). The composition of ambient HULIS is not well known, but limited studies (Sannigrahi et al., 2006; Sullivan and Weber, 2006a, b), have identified it to be rich in large molecular weight compounds, and compounds of aromatic moieties, of which quinones and hydroxyquinones are a subset. However, the detailed chemical speciation of HULIS and the identification of its ROS-active components have not been done.

In this study, we present a technique to fractionate ambient HULIS based on the differential elution of its various components in organic solvents of varying polarity. Sub-fractions were eluted sequentially in hexane, dichloromethane (DCM) and methanol, and analyzed separately for the DTT assay to identify the fractions most strongly associated with ROS generation. The contribution of quinones in the water-soluble DTT activity of ambient PM in the southeast US was also assessed by two mechanistic approaches – first by measuring their atmospheric concentration, second by comparing their elution behavior with ambient HULIS in the fractionation scheme. Although, the HULIS fractionation technique is applied in the context of the DTT assay here, we anticipate it to be adaptable to other toxicity probes, and by combining with detailed chemical analysis of various sub-fractions, it could present a potentially valuable tool to narrow-down the major functional groups of ambient PM associated with ROS generation, and possibly adverse health effects.

2. Experimental methods

2.1. Sampling methods and locations

Ambient $PM_{2.5}$ particles were collected on pre-baked (550 °C for 12 h) quartz filters ($8 \times 10''$, Pallflex® Tissuquartz™, Pall Life Sciences) for 23 h using a high volume (HiVol) sampler (Thermo Anderson) with volumetric flow control and $PM_{2.5}$ separation via impaction, operating at a nominal flow rate of 1.13 m^3/min. Sampling was conducted at four different sites in and around Atlanta. The Jefferson Street (JST) site was operated as a fixed urban site throughout the sampling period. A mobile trailer containing the sampling instruments was moved amongst three other sites, Yorkville (YRK), Georgia Tech (GT) and roadside (RS), over the course of a year (June 2012–May 2013). JST, located approximately 8 km from Atlanta's city center in an urban environment, is isolated from direct emissions. Aged urban vehicular and industrial emissions are presumably the major sources contributing to this site. Ambient data from JST has been the basis for many health studies from the metropolitan region (Darrow et al., 2014; Strickland et al., 2009; Tolbert et al., 2000). The RS site is adjacent to a major freeway (I-85/75), where the dominant sources of the particles are expected to be some diesel, but predominately gasoline exhaust emissions. The GT site was on the rooftop of our departmental building (Ford ES&T) at the Georgia Institute of Technology, situated at an elevation of 20–40 m above ground level (front vs. back of building). It is an intermediate site between JST and RS, providing data impacted somewhat by roadway emissions. YRK, a rural site located about 70 km northwest of Atlanta, is primarily influenced by biogenic and agricultural emissions in the area. The closest residences are ~1 km from this site. A map of the sampling sites is included in the