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Article

Environmentally Persistent Free Radicals, Reactive Oxygen Species Generation, and Oxidative Potential of Highway $PM_{2.5}$

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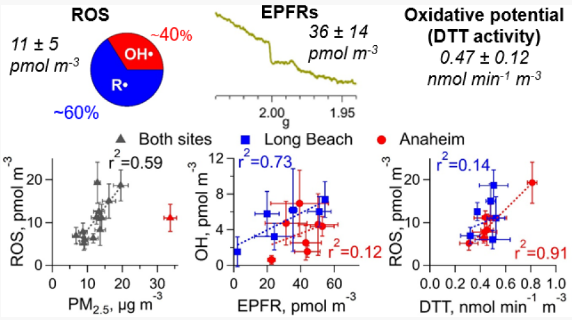
ABSTRACT: In urban environments, vehicle exhaust and nonexhaust emissions represent important sources of fine particulate matter with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$), which plays a central role in adverse health effects and oxidative stress. We collected $PM_{2.5}$ filter samples from two highway sites (Anaheim and Long Beach, CA) and an urban site (Irvine, CA) to quantify environmentally persistent free radicals (EPFRs) contained in $PM_{2.5}$ and the generation of radical forms of reactive oxygen species (ROS) in water using electron paramagnetic resonance spectroscopy. The EPFR concentrations were 36 ± 14 pmol m^{-3} at highway sites, which were about two times higher than those at the urban site. EPFRs correlate positively well with CO, NOx, and elemental and organic carbon, indicating that EPFRs are emitted from vehicular exhaust. Good correlations of EPFRs and Fe and Cu may indicate that EPFRs are stabilized by Fe and Cu emitted from tire and brake wears. EPFRs are negatively correlated with ozone, suggesting that photochemistry does not play a large role in the formation of EPFRs and possibly also indicating that EPFRs are quenched by ozone. Highway $PM_{2.5}$ are found to generate mainly OH and organic radicals in the aqueous phase. The generated ROS are correlated with $PM_{2.5}$ mass concentrations and OH radicals show a good correlation with EPFRs, implying the role of EPFRs in aqueous OH radical generation. The $PM_{2.5}$ oxidative potentials as quantified with the dithiothreitol (DTT) assay are correlated with ROS, OH, and organic radicals for $PM_{2.5}$ collected in Anaheim, whereas little correlations are observed for Long Beach. These findings highlight the interplay of various PM redox-active chemical components and complex relationship between ROS formation and DTT activity.



KEYWORDS: *environmentally persistent free radicals, reactive oxygen species, DTT activity, vehicular exhaust and nonexhaust emissions, traffic suspended particles*

INTRODUCTION

Traffic-related emissions are one of the most important sources of particulate matter (PM) in urban environments. Exposure to the traffic-related fine PM is often associated with adverse health effects and oxidative stress.[1−5] Within the past two decades, many countries have implemented stringent regulations leading to a significant reduction in exhaust or tailpipe emissions and PM mass concentrations.[6−8] In contrast, nonexhaust emissions from brakes, tires, and road wear are currently not regulated, but their importance in urban air quality has been increasingly recognized.[8−11] Correlation analyses from recent studies suggest that nonexhaust emissions also contribute to fine PM toxicity, highlighting the importance of investigating relations among sources, chemical composition, and particle toxicity.[9,10] Recent epidemiological studies have shown strong associations of long-term exposure to nontailpipe emissions with respiratory and cardiovascular diseases.[12,13]

Ambient PM contains particle-bound free radicals, so-called environmentally persistent free radicals (EPFRs); in contrast to common free radicals with short lifetimes, EPFRs in atmospheric particles can persist for days or longer.[14−17] EPFRs may originate from both primary and secondary sources. Previous studies have found that particles emitted from coal combustion, traffic, and dust contain large amounts of EPFRs.[18−20] The chemical identity of EPFRs generated from incomplete combustion can be semiquinone, phenoxyl, and cyclopentadienyl radicals formed by thermal decomposition of organic precursors on metal-containing particles.[21,22] EPFRs can also be formed in secondary processes such as heterogeneous oxidation of polycyclic aromatic hydrocarbons (PAHs) by ozone[23,24] and oxidation of

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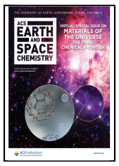

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naphthalene followed by the formation of secondary organic aerosols (SOA).[25]

PM toxicity is related to its ability to cause oxidative stress[26−28] by the formation of reactive oxygen species (ROS) upon inhalation and respiratory deposition of PM.[29,30] ROS include hydroxyl radical (OH·), superoxide radical ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and organic radicals, which can deplete antioxidants and induce oxidative damage to lipids and tissues.[12,30−32] PM contains redox-active components including quinones and transition metals,[33−36] which can generate ROS via Fenton(-like) reactions, quinoid redox cycling, and decomposition of organic hydroperoxides.[25,37−41] EPFRs are also shown to catalytically generate ROS by redox reactions.[37,38,42]

Oxidative potential of PM represents the redox activity of particles to generate ROS. One of the most commonly used methods to quantify PM oxidative potential is the dithiothreitol (DTT) assay.[43−45] This method is sensitive to quinones,[46] transition metals,[45,47,48] humic-like substances (HULIS)[49,50] and it has also successfully applied to SOA formed by oxidation of naphthalene,[25,51−53] toluene,[54] and isoprene SOA.[49,50,55] Both exhaust and nonexhaust emissions have been identified as important sources contributing to PM DTT activities.[56−61] Studies have shown associations of DTT activities with various health outcomes,[47,62−64] suggesting that controlling oxidative potential would add another dimension to the regulation of aerosol health effects in addition to reducing PM mass concentrations.[60] Note that DTT is not a physiological antioxidant in the human body and the robustness of the DTT assay in predicting PM health effects is still to be established. The similarity in PM chemical contributors to EPFRs, ROS generation, and DTT activities implies possibly close associations among them, but the link and interplay among them for causing oxidative stress are still unclear and warrant further investigations.

Traffic-related emissions contain high levels of EPFRs, quinones, and transition metals, providing a good source to study such associations to understand the contribution from exhaust and nonexhaust emissions. In this study, ambient PM with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) was collected using a high-volume $PM_{2.5}$ sampler at highway sites in Anaheim and Long Beach as well as at an urban site in Irvine (all sites in Southern California, U.S.). The past chemical analyses at these locations have shown that the vehicle emissions cause poor air quality.[9,10] Particle-bound EPFRs were measured using a continuous-wave electron paramagnetic resonance (CW-EPR) spectrometer. The generation of ROS was quantified by extracting the particles in water and analyzing the extracts using EPR coupled with a spin-trapping technique. The DTT activity was measured by monitoring the consumption of DTT over time in $PM_{2.5}$ total extracts including the water-soluble and -insoluble fractions (hereafter called "total DTT activity"). We measured PM chemical components including organic carbon (OC), elemental carbon (EC), and transition metals. Gaseous pollutants including ozone (O_3), nitrogen monoxide (NO), nitrogen dioxide (NO_2), and carbon monoxide (CO) were also obtained. The correlations of EPFRs, ROS generation, and total DTT activity as well as $PM_{2.5}$ mass concentration were investigated for better understanding of aerosol health effects and oxidative stress by PM from traffic-related emissions.

■ MATERIALS AND METHODS

PM Collection. A high-volume sampler (Hi-Vol, Tisch Environmental, flow rate 1.13 m^3 min^{-1}) was used to collect ambient $PM_{2.5}$ onto prebaked 8" × 10" microquartz filters at an urban site (a campus building at the University of California, Irvine, 33°38′40.4″N 117°50′39.3″W, elevation 20 m) and two highway sites in Anaheim (Interstate 5, 33°49′09.4″N 117°55′07.5″W) and Long Beach (Interstate 710, 33°51′34.0″N 118°12′01.0″W) within 20 m from the highway roads. The Interstate 5 (I-5) is one of the busiest highways in Southern California and known as a commuter route from Orange County to the city of Los Angeles. The other highway site, Interstate 710 (I-710), is a major route that connects Long Beach and the Los Angeles city with a higher fraction of heavy-duty vehicles and known as heavy-duty corridor.[10] Particle filter samples were collected for 4−12 h daily from 1/28/2020 to 2/3/2020 at the Anaheim site, for 6−12 h per day from 2/4/2020 to 2/10/2020 (except on 2/9/2020) at the Long beach site, and for 8−11 h at the urban site from 2/23/2020 to 2/29/2020 (except on 2/28/2020). Field blanks were collected every other day at each site. After the collection, all filters were wrapped in a prebaked aluminum foil and immediately stored in a freezer (−18 °C). Portions of the filters collected from the highway sites were cut using a circular punch with a diameter of 2.54 cm and used for measurement of EPFRs, ROS, and DTT activities. Those from the urban sites were analyzed for EPFRs. Measurements of EPFRs, ROS, and DTT on each filter were repeated 3−5 times. EPFR measurements were conducted within 2 weeks of sample collection, and ROS and DTT measurements were conducted within 4−6 months. Note that EPFRs are stable at least a year after collection (Figure S1). Additionally, $PM_{2.5}$ samples were collected on one 47 mm Teflon-membrane filter and two 47 mm quartz-fiber filters in parallel using a medium-volume sampler with a Bendix Model 240 $PM_{2.5}$ cyclone at both highway sites. These filters were used to determine PM mass, metals, OC, and EC at the highway sites. The sampling flow rate was 37.7 L min^{-1} for each filter channel and the sampling duration was 4 h covering the following periods: 6:00−10:00, 10:00−14:00, and 14:00−18:00. The total sampling periods overlapped with the Hi-Vol sampling periods. The Teflon-membrane filters were analyzed for $PM_{2.5}$ mass and metal while the quartz fiber filters were analyzed for OC and EC.[65]

Environmentally Persistent Free Radicals (EPFRs). One or two circular punches from each filter sample were inserted into a quartz tube (9.17 mm I.D., SP Wilmad-LabGlass) for EPFR measurements using a CW-EPR spectrometer (EMXplus, Bruker, Germany). The following parameters were used in EPR: a microwave frequency of 9.65 GHz; a microwave power of 36.18 mW (8 dB); a modulation frequency of 100 kHz; a modulation amplitude of 1.0 G; a receiver gain of 40 dB; a time constant of 10.24 ms; and a magnetic field scan of 1623.06 G. Ambient concentrations of EPFRs are presented as EPFR per volume of air (EPFRv, pmol m^{-3}) and per mass of $PM_{2.5}$ (EPFRm, pmol μg^{-1}). Paramagnetic species are characterized based on their *g*-factor values: free electrons have a *g*-factor value of 2.0023 and organic radicals have slightly higher values depending on the number of oxygen atoms in the molecule.[21]

Reactive Oxygen Species (ROS) Generation. EPR combined with a spin-trapping technique was applied to detect radicals in the aqueous particle extracts. Two circular

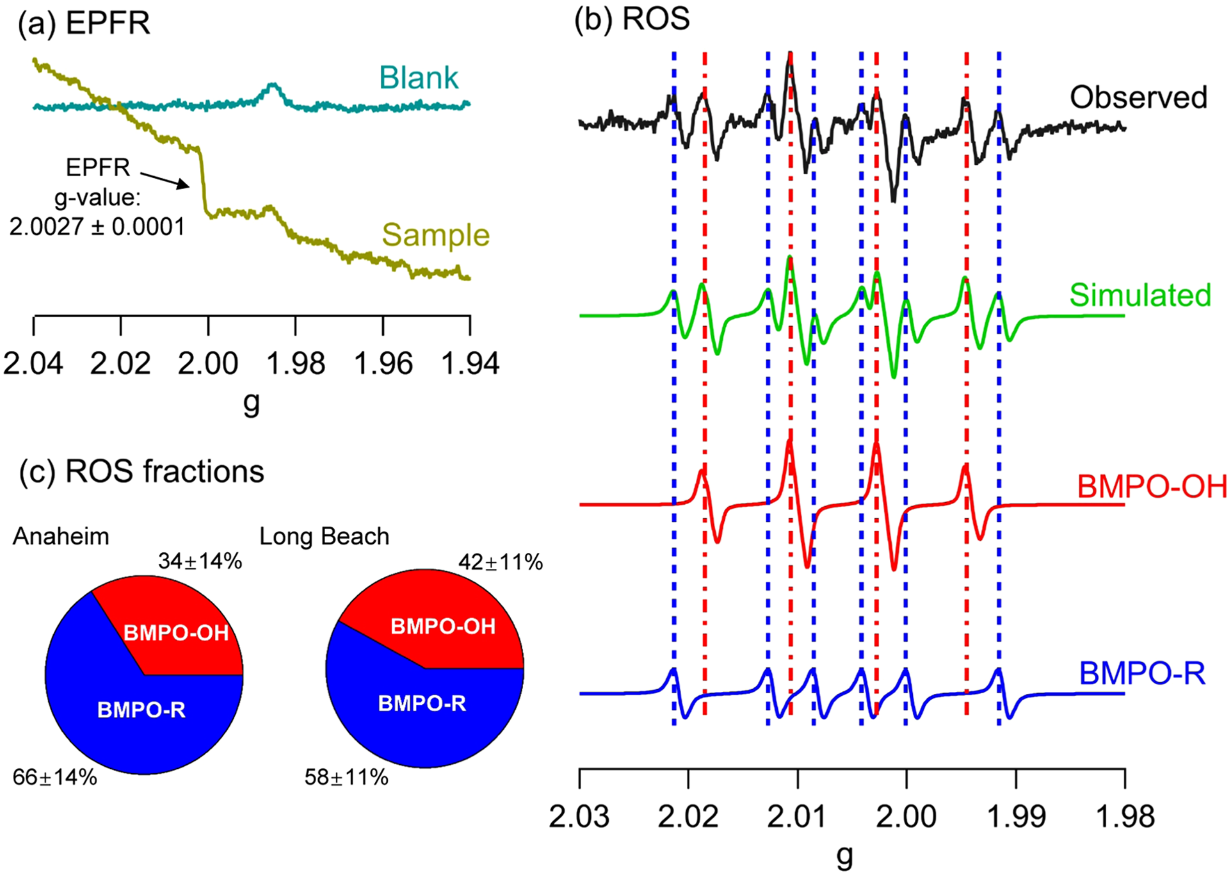

Figure 1. (a) Typical EPR spectra of a quartz blank filter and ambient $PM_{2.5}$ containing EPFRs. (b) The observed EPR spectrum (black) of the aqueous extracts of $PM_{2.5}$ collected at a highway site and the simulated spectrum (green) by deconvolution into OH radicals (red) and carbon-centered radicals (blue) trapped by spin-trapping agent BMPO. The vertical dashed lines indicate the position of each peak for BMPO−OH and BMPO−R adducts. (c) Averaged fractions of OH and carbon-centered radicals (R) as trapped by BMPO in aqueous extracts of $PM_{2.5}$ collected at highway sites in Anaheim and Long Beach.

punches from each filter sample were extracted in 1.3 mL of 10 mM spin-trapping agent 5-tert-butoxycarbonyl-5-methyl-1-pyrroline-N-oxide (BMPO) in Millipore water (>18 Ω cm^{-1}) for 8−10 min using an analog vortex mixer (VWR International LLC) and both water-soluble and insoluble compounds were extracted. The extracts were then concentrated 5−20 times through blowing under N_2 gas for 10−13 min. The remaining solution was inserted into a 50 μL micropipette and measured within 25 min from extraction. The EPR parameters used for ROS measurement were the same as those for EPFRs except for the following: a microwave frequency of 9.86 GHz, a microwave power of 21.17 mW (10 dB); a time constant of 20.48 ms; a modulation amplitude of 2.0 G; and a magnetic field scan of 150.0 G. The SpinFit and SpinCount modules in the Xenon software were applied to simulate each EPR spectrum to identify and quantify different radical adducts in the extracts. Data are normalized to the sampled volume of air (ROSv, pmol m^{-3}) and $PM_{2.5}$ mass (ROSm, pmol μg^{-1}).

Total Dithiothreitol (DTT) Activities. Two circular punches from each filter sample were extracted in 7 mL of Millipore water for 8−10 min using the vortex mixer. The DTT assay was conducted on the extracts and the filter following the same DTT protocol in Gao et al. (2017).[66] The measured total DTT activities include the contributions from both the water-soluble and water-insoluble fractions. In brief, 7 mL of the extracts and the filter were incubated at 37 °C with 2 mL of potassium phosphate buffer and 1 mL of DTT (1 mM) and shaken continuously in a ThermoMixer (Eppendorf North America, Inc.). At specified time points (5, 10,15, 20, 25 min), a small aliquot (100 μL) of the incubated mixture was withdrawn and mixed with 1 mL of trichloroacetic acid to quench the consumption of DTT. The quenched mixture was further mixed with 2 mL of Tris buffer (0.08 M with 4 mM EDTA (ethylenediaminetetraacetate)) and 0.5 mL of DTNB (0.2 mM, 5,5′-dithiobis(2-nitrobenzoic acid)). One milliliter of this mixture was diluted with 9.5 mL of water and filtered using a 0.22 μm pore syringe filter (Millex). The filtered solution was immediately measured for absorbance at 412 and 700 nm wavelength using the liquid waveguide capillary cell with an optical path length of 100 cm (World Precision Instruments, Inc.) coupled to the ultraviolet−visible spectrophotometer (DH-MINI, Ocean Optics, Inc.) and the multiwavelength light detector (FLAME-T-UV−vis-ES, Ocean Optics, Inc.). The total DTT activities were calculated from the linear regression of absorbance versus time and presented as the total DTT activities per volume of air (total DTTv, pmol min^{-1} m^{-3}) and per mass of $PM_{2.5}$ (total DTTm, pmol min^{-1} μg^{-1}).[67]

Gaseous Pollutants. Real-time online measurements of gaseous pollutants were conducted at both sites. CO was measured with the Teledyne Model 300E CO analyzer every minute. NO and NO_2 were measured with an ECO Physics CLD 60 analyzer. Hourly O_3 was obtained from SCAQMD Long Beach - Signal Hill (33°47′37.4″N 118°10′15.7″W) and

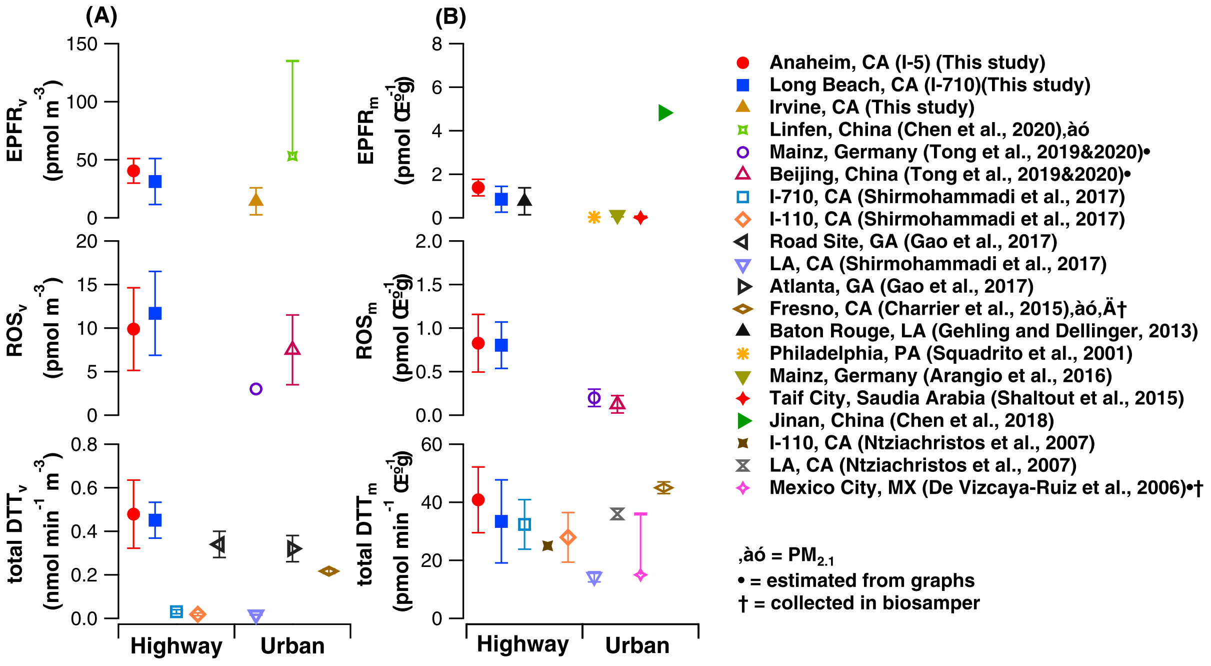

Figure 2. Air-volume- and $PM_{2.5}$-mass-normalized concentrations of EPFRs and ROS as well as total DTT activities of $PM_{2.5}$ collected at two highway sites and an urban site, CA, in comparison to ambient data from previous studies including Squadrito et al. (2001),[92] De Vizcaya-Ruiz et al. (2006),[93] Ntziachristos et al. (2007),[94] Gehling and Dellinger (2013),[17] Shaltout et al. (2015),[95] Charrier et al. (2015),[91] Arangio et al. (2016),[15] Shirmohammadi et al. (2017),[10] Gao et al. (2017),[66] Chen et al. (2018),[20] Tong et al. (2019),[40] Tong et al. (2020),[88] and Chen et al. (2020).[77] Data in this study were averaged concentrations with error bars representing daily variability calculated from the standard deviations of the averaged data. Data from winter seasons (November to March) from previous studies were selected for comparisons. Markers with bars (Linfen, Mexico City) denote the range of ambient concentrations and markers with error bars are either reported values or estimated from previous studies.

Anaheim - Loara School (33°49′50.2″N 117°56′18.6″W) sites (https://ww2.arb.ca.gov/).

$PM_{2.5}$ Mass and Metals. Blank and exposed Teflon-membrane filters for the medium-volume sampler were equilibrated in a clean room with controlled temperature (21.5 ± 1.5 °C) and relative humidity (35 ± 5%) for >24 h before mass determination by gravimetry. Filters were weighed before and after sampling with a ±1 μg sensitivity microbalance (XP6, Mettler Toledo, LLC) to obtain gravimetric $PM_{2.5}$ mass.[68] Teflon-membrane filters were then analyzed for 51 elements (from sodium to uranium) by high sensitivity energy-dispersive X-ray fluorescence (XRF, Panalytical Epsilon 5).[69] Iron (Fe) and copper (Cu) are selected to discuss in this work, as they are known to be redox-active to cause ROS formation.

OC and EC. OC and EC were quantified from the quartz-fiber filters collected by the medium-volume sampler. The analysis followed the IMPROVE_A thermal/optical protocol using a multiwavelength OC/EC analyzer (DRI Model 2015, Magee Scientific).[70−72]

■ RESULTS AND DISCUSSION

Environmentally Persistent Free Radicals. Figure 1a shows an example of EPR spectrum of the $PM_{2.5}$ particles and a blank filter. The EPR spectrum of ambient $PM_{2.5}$ particles exhibits a large signal with a *g*-factor of 2.0027 ± 0.0001 and a peak-to-peak distance of 4.4 ± 0.7 G, which are consistent with previously reported values for EPFRs and characteristics for semiquinone-type radicals.[15,41,73] The drift in the spectrum in particle samples as opposed to the flat spectrum of the blank filters is likely due to the presence of transition metals.[38,74] Measurements of the filter after roughly one year of storage at −18 °C show that the signals were almost unchanged, indicating the persistency of EPFRs (Figure S1). This is consistent with previous studies showing that some portions of EPFRs have a lifetime longer than months to indefinite[17] as semiquinone-type radicals can be stabilized by their adsorption into a polymeric carbonaceous core of PM[75] or by electron transfer with transition metals.[14] In addition, a very recent study demonstrated the formation and stabilization of carbon-centered radicals in organic aerosols containing Fe ions.[76]

Figure 2 shows the average EPFRv and EPFRm concentrations at the highway and urban sites in comparison to those from other studies using the same analytical method. The two highway sites have similar levels of EPFRv (40 ± 11 and 31 ± 20 pmol m^{-3} at Anaheim and Long Beach, respectively) and are more than twice higher than EPFRv at the urban site (14 ± 12 pmol m^{-3}). The EPFRm at Anaheim (3.5 ± 1.0 pmol μg^{-1}) is higher than that at Long Beach (2.1 ± 1.4 pmol μg^{-1}). Compared to reported values on EPFR concentrations at near-road and urban environments in the U.S., Europe, and Asia, EPFRs at near-road sites are generally higher than those from urban background environments, except two studies reporting high levels of EPFRs in Linfen and Jinan, highly polluted urban cities in China.[20,77] This suggests that EPFRs are likely associated with traffic-related emissions, consistent with many earlier studies.[22,73,77,78]

Figure 3 shows the correlation between EPFRv and gaseous pollutants including CO, NO, NO_2, and O_3 as well as $PM_{2.5}$ components including OC, EC, and metals (Fe, Cu) at both

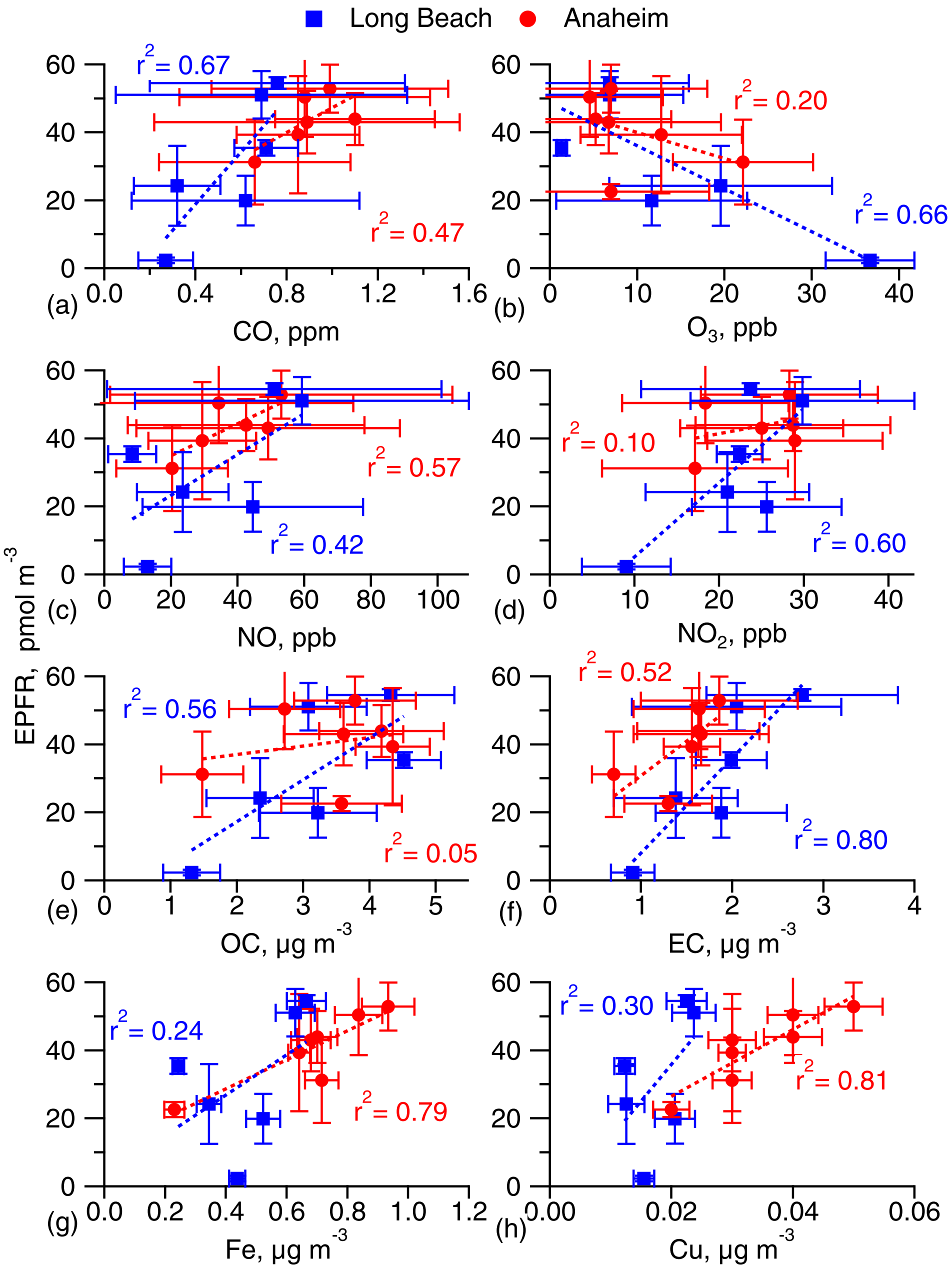

Figure 3. Correlations between volume-normalized EPFR concentrations and gaseous pollutants (CO, O_3, NO, NO_2) and chemical components (OC, EC, Fe, Cu) measured at the Anaheim and Long Beach highway sites. The error bars on EPFRs represent the standard deviations of multiple measurements. The error bars on gaseous pollutants are standard deviations of online measurements. The error bars for OC, EC, and metal measurements represent variability of their ambient concentrations and also are propagated from analytical and sampling volume uncertainties in these measurements.

highway sites. A summary of correlation coefficients and *p*-values are given in Table S2 and S3. Note that the error bars on chemical species reflect the viability in their ambient concentrations which are averaged over the sampling time (e.g., 12 h) of the $PM_{2.5}$ sampler. For both sites, EPFRs show good positive correlations with a combustion marker CO and a traffic-emission marker NO. The strong positive correlations of EPFRs with soot particle EC suggest that EPFRs are likely associated with diesel exhaust particles.[79] A recent study showed that EPFRs measured from the $PM_{3.3}$ collected in China have a positive correlation with EC in winter, and their source-receptor modeling suggested that EPFRs are mainly emitted from coal combustion.[77] In our study, the EC to TC (total carbon) ratio is slightly higher at the Long Beach site (0.37 ± 0.03) compared to the Anaheim site (0.31 ± 0.04), while the EC to CO ratio is much higher at the Long Beach site (3.4 ± 0.6 $\mu g\ m^{-3}\ ppm^{-1}$) than the Anaheim site (1.7 ± 0.3 $\mu g\ m^{-3}\ ppm^{-1}$). Diesel engines are known to emit less CO and more black carbon (BC), yielding higher EC fractions and EC to CO ratios.[80] The higher EC fractions and EC to CO ratios at the Long Beach site are consistent with the I-710 freeway serving as a major corridor accommodating a high volume of heavy-duty diesel trucks daily, while the I-5 freeway (Anaheim) has mainly light-duty vehicles fleet.[81]

At the Anaheim site, EPFRs are strongly correlated with Fe ($r^2 = 0.79$) and Cu ($r^2 = 0.81$). Fe and Cu are important metals

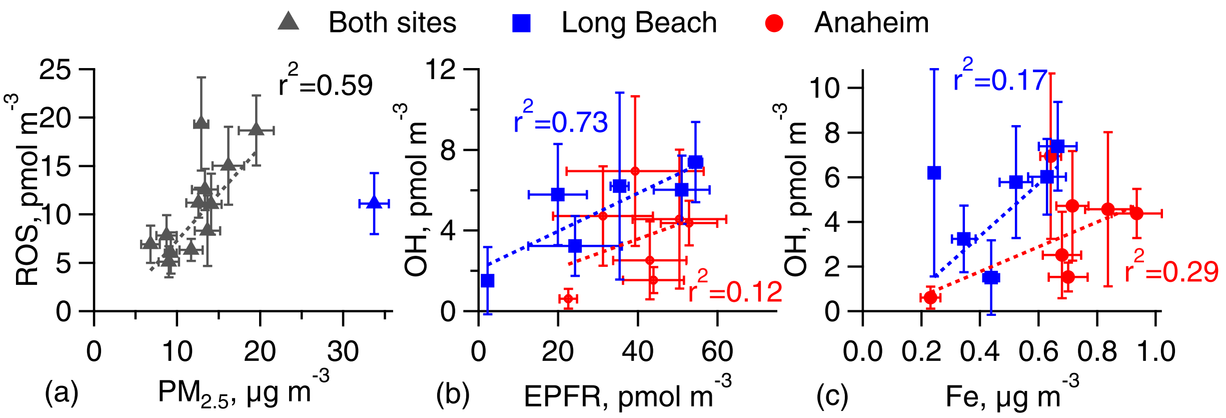

Figure 4. Correlations between (a) radical forms of ROS in the aqueous $PM_{2.5}$ extracts and $PM_{2.5}$ mass concentrations, (b) OH radicals in the aqueous $PM_{2.5}$ extracts and EPFR, and (c) OH radicals in the aqueous PM extracts and Fe. A red triangle in panel a denotes the measurement on Feb. 8, 2020 at Long Beach, which is considered as an outliner (see text). The error bars on ROS, OH, and EPFR represent the standard deviations of multiple measurements, and those on $PM_{2.5}$ and Fe are propagated from analytical and sampling volume uncertainties in these measurements.

emitted from tire or brake wears.[82] Both metals are higher at the Anaheim site than the Long Beach site (Table S1). Studies have shown that EPFRs are often associated with metals during combustion processes.[21,22] PAHs can be oxidized and chemisorbed to the surface of metal particles to form EPFRs on the surface of particles.[14,73] A recent study on chemical measurements on tire-wear particles showed that toxic quinones can be found in the tire-wear extracts.[83] The strong correlation of EPFRs with these metals may suggest that EPFRs at the Anaheim site are stabilized by interacting with transition metals such as Fe and Cu under ambient conditions.

The EPFRs measured at both highway sites show negative correlation with O_3, which may be stemmed from a negative correlation of O_3 with NO owing to the O_3 titration by NO (as NO correlates positively with EPFR). It may indicate that EPFRs are quenched by O_3 to form closed-shell organic compounds, which is in line with previous computational simulations suggesting that stabilized Criegee intermediates can react with ozone to form stable organic compounds.[84] It is however in contrast to previous studies showing that heterogeneous ozonolysis of PAHs can lead to the formation of persistent radicals.[23,24] Weak positive relationships of EPFRs with solar radiation were observed previously[17] and a recent study also demonstrated that visible-light exposure of EPFRs contained in $PM_{2.5}$ can enhance the EPFR concentration and generate more oxidized organic radicals.[41] Laboratory experiments have shown that EPFRs formed from ozonolysis of PAHs decayed upon exposure to elevated concentrations of NO_2,[24] but negative correlations of EPFR and NO_2 are not observed in this study. A previous study has shown that the lifetime of EPFRs contained in naphthalene SOA is longer under dry conditions,[25] but we do not observe a correlation between EPFRs and relative humidity ($r^2 \leq 0.18$, Figure S2). There may be complex competing effects for the fate of EPFRs by solar radiation, ambient conditions, and interactions with oxidants. Although our observations suggest that photochemical and secondary processes may not play a large role in the formation of EPFRs at these two highway sites, further laboratory studies are warranted to investigate interactions of EPFRs with gaseous pollutants to gain mechanistic understanding.

ROS Generation in Water and Relations with EPFRs and $PM_{2.5}$ Mass. Figure 1b shows an example of the observed EPR spectrum (black) of the aqueous extracts of $PM_{2.5}$ collected at the Anaheim site. The EPR spectrum is composed of several peaks, indicating contributions from different types of radicals. The simulated EPR spectrum (green) reproduced the observed spectrum (black) very well and can be deconvoluted into four- and six-line signals due to the hyperfine splitting of BMPO−OH and BMPO−R adducts, respectively. It indicates that ambient $PM_{2.5}$ collected from the two highway sites form mainly OH and carbon-centered organic radicals upon interaction with water. Note that Fe(III) is known to withdraw an electron from nitric oxide spin trap and induce positive BMPO−OH artifacts.[85,86] However, Fe(III) in atmospheric particles is likely largely complexed with organic ligands,[87] and our experiments show that complexation leads to the stark suppression of BMPO−OH signals from Fe(III) (Figure S3); hence, such artifacts are likely to be minimum in our measurements.

The fraction of OH and carbon-centered radicals to total ROS is approximately 40% and 60%, respectively (Figure 1c). Arangio et al. (2016) reported that OH and carbon-centered radicals contributed 10−87% and 9−62% of total radical forms of ROS, respectively, in Mainz, Germany.[15] Tong et al. also found large contributions from OH (20−50%) and organic radicals (35−80%) in ambient particles in multiple cities.[40,88] ROSv and ROSm concentrations are similar at both highway sites to be ∼10 (±5) pmol m^{-3} and 0.8 (±0.2) pmol μg^{-1}, respectively (Figure 2). Comparison of reported values from other studies shows that ROSv and ROSm from highway sites are higher than urban environments, suggesting that traffic-related emissions play an important role in ROS formation, although more studies on different locations are needed to provide better statistics.

Figure 4a shows that total ROSv concentrations have a moderate positive correlation with $PM_{2.5}$ mass concentrations ($r^2 = 0.59$) at both highway sites with the exclusion of one outliner from the measurement on 2/8/2020 at the Long Beach site (red triangle). This day had the highest relative humidity (RH = 95%) and was the only foggy day recorded during the campaign. The $PM_{2.5}$ concentration on this day reached up to 35 $\mu g\ m^{-3}$, which was much higher than the other sampling days with mass concentrations less than 20 $\mu g\ m^{-3}$, while the ROS concentration on this day was within the range of the other sampling days. $PM_{2.5}$ nitrate was the

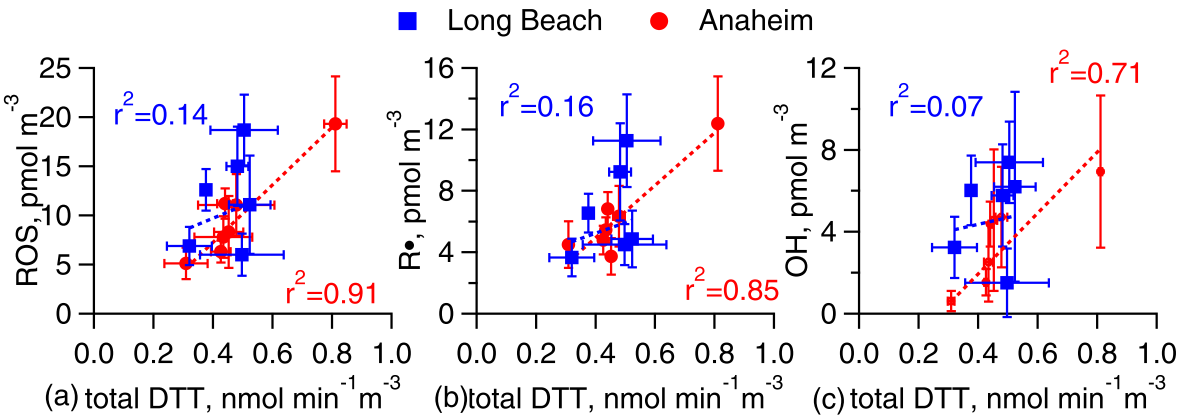

Figure 5. Correlation of total DTT activities with concentrations of (a) radical forms of ROS, (b) carbon-centered radicals (R·), and (c) OH radicals formed in aqueous extracts of $PM_{2.5}$ collected at the Anaheim and Long Beach sites.

chemical composition with the largest increase (13.5 μg m^{-3}) on that day. It is likely that the foggy environment provided a favorable condition for aqueous chemistry to produce chemical compounds that add to the mass but did not contribute to ROS generation.[89] A recent study found that ROSv generated from fine PM showed a positive correlation with PM mass concentrations at forest sites (Amazon and Hyytiälä) and urban cities in China (Beijing, Guangzhou, Shanghai, and Xi'an).[40] Another study found that the superoxide generated from fine PM in Beijing also showed a positive correlation with $PM_{2.5}$ mass concentration in winter.[90]

Figure 4b shows that OH radicals have a positive strong correlation with the EPFRs at the Long Beach site (r^2 = 0.73). OH radicals are also positively correlated with primary vehicle exhaust source markers including CO and EC, and negatively correlated with O_3 (Figure S4). This may suggest that OH radicals stem from the similar sources as EPFRs or are generated from EPFRs. In fact, past studies have shown that EPFR-containing combustion generated particles and atmospheric $PM_{2.5}$ can generate OH radicals in aqueous solutions.[37,38,42] OH radicals and EPFRs at the Anaheim site also show a positive but much weaker (r^2 = 0.12) correlation, indicating that additional sources such as Fenton(-like) reactions and aqueous-phase decomposition of organic hydroperoxide[85] may contribute to OH formation at the Anaheim site. A slightly higher correlation between Fe and OH radicals is observed at the Anaheim site (r^2 = 0.29) compared to the Long Beach site (r^2 = 0.17) (Figure 4c).

DTT Activities and Relation with ROS Generation. The two highway sites have similar levels of total DTT activities (Figure 2) and the average total DTTv and DTTm are ~0.47 nmol min^{-1} m^{-3} and ~37 pmol min^{-1} μg^{-1}, respectively. These values are similar or slightly higher than previous measurements at highway or road sites.[10,66,91] Interestingly, the total DTTv activities at the Anaheim site are strongly correlated with ROSv (r^2 = 0.91, Figure 5a). For individual ROS species, the correlations of total DTTv with organic radicals (r^2 = 0.85, Figure 5b) are stronger than OH radicals (r^2 = 0.71, Figure 5c), suggesting that the DTT decay and the formation of organic radials are contributed by similar organic compounds. This is consistent with a recent study reporting that organic hydroperoxides can consume DTT.[54] The lower correlation of total DTTv with OH radicals is expected as the DTT assay is less sensitive to Fe, which plays a large role in generating OH radicals through Fenton chemistry.[30] There is little correlation between total DTTv and ROSv or individual species at the Long Beach site ($r^2 \leq$ 0.16, Figure 5).

A recent study found that secondary inorganic components, crustal material, and biogenic secondary organic aerosols control PM mass concentrations, while oxidative potential is associated mostly with anthropogenic sources in particular with residential biomass burning and metals from vehicular nonexhaust emissions.[60] Our previous study applied kinetic modeling[29] to estimate ROS formation from measured chemical components including OC, metals, and quinones, and the results showed that the measured water-soluble DTT activities are correlated with the modeled formation rates of H_2O_2 and superoxide radicals.[30] The mixed results in this study provide promising but cautious implications from ambient data that DTT activities could represent ROS generation, but further studies are necessary to improve a mechanistic understanding of associations among DTT activities, ROS formation, and oxidative stress. Other OP assays such as ascorbic (AA) or glutathione (GSH) assays that measure the consumption of antioxidants could also be used to assess the relations of OP with ROS generation in future studies.

■ CONCLUSIONS

Exhaust and nonexhaust emissions contribute to ambient fine particulate matter, posing health risks. We collected $PM_{2.5}$ particles from highway and urban sites in Southern California and measured EPFRs, ROS generation, and total DTT activities. We find that $PM_{2.5}$ at highway sites contain substantial amounts of EPFRs with about two times higher concentrations than the urban site. Highway $PM_{2.5}$ is found to mainly generate OH and carbon-centered radicals upon extraction into water. Positive correlations of EPFRs with vehicle exhaust markers (CO, NO, and EC) and nonexhaust markers (Fe and Cu) suggest that EPFRs are associated with diesel exhaust particles and that they are semiquinone-type radicals stabilized by transition metals. Negative correlations of EPFRs with O_3 suggest the minor role of photochemistry on EPFR formation. We also find interesting links among EPFRs, ROS generation, and total DTT activities at the highway sites. EPFRs show a strong positive correlation with aqueous OH radical formation at one highway site, suggesting that EPFRs are redox-active to generate OH radicals in water. The atmospheric persistency of EPFRs and their ability to generate

OH radicals make them highly relevant in studying the health impacts of highway emissions. The formation of radical forms of ROS is strongly correlated with $PM_{2.5}$ mass concentrations and total DTT activities at one highway site, providing rationale of the use of oxidative potential as an additional indicator of PM toxicity other than PM mass concentrations. As such a correlation is weak at another site with different sources, further measurements with a longer period of sampling times and also at various locations with different sources are necessary to better understand the associations of EPFRs, ROS generation, and DTT activities with oxidative stress induced by atmospheric particulate matter and its adverse effects on human health.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsearthspacechem.1c00135.

> Average concentrations of OC, EC, O_3, NO, NO_2, CO, and metals; correlation coefficient (r^2) and p-value of fine particle OP, ROS, and DTT activities with $PM_{2.5}$ mass and selected chemical species; EPR spectra of $PM_{2.5}$ to showing stability of EPFRs; correlation of EPFRs and relative humidity; EPR spectra of mixtures of BMPO and Fe(III) as well as BMPO, Fe(III), and oxalic acid (OA); correlation of OH with EC, OC, CO, and O_3, including Tables S1–S3 and Figures S1–S4 (PDF)

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Notes

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