Water Research 102 (2016) 241–251

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

A non-target approach to identify disinfection byproducts of structurally similar sulfonamide antibiotics

Mian Wang, Damian E. Helbling*

School of Civil and Environmental Engineering, Cornell University, Ithaca, NY, USA

ARTICLE INFO

Article history:
Received 5 March 2016
Received in revised form
20 May 2016
Accepted 18 June 2016
Available online 23 June 2016

Keywords:
Sulfonamide antibiotic
Disinfection byproducts
High-resolution mass spectrometry
Transformation products
Non-target analysis

ABSTRACT

There is growing concern over the formation of new types of disinfection byproducts (DBPs) from pharmaceuticals and other emerging contaminants during drinking water production. Free chlorine is a widely used disinfectant that reacts non-selectively with organic molecules to form a variety of byproducts. In this research, we aimed to investigate the DBPs formed from three structurally similar sulfonamide antibiotics (sulfamethoxazole, sulfathiazole, and sulfadimethoxine) to determine how chemical structure influences the types of chlorination reactions observed. We conducted free chlorination experiments and developed a non-target approach to extract masses from the experimental dataset that represent the masses of candidate DBPs. Structures were assigned to the candidate DBPs based on analytical data and knowledge of chlorine chemistry. Confidence levels were assigned to each proposed structure according to conventions in the field. In total, 11, 12, and 15 DBP structures were proposed for sulfamethoxazole, sulfathiazole, and sulfadimethoxine, respectively. The structures of the products suggest a variety of reaction types including chlorine substitution, S–C cleavage, S–N hydrolysis, desulfonation, oxidation/hydroxylation, and conjugation reactions. Some reaction types were common to all of the sulfonamide antibiotics, but unique reaction types were also observed for each sulfonamide antibiotic suggesting that selective prediction of DBP structures of other sulfonamide antibiotics based on chemical structure is unlikely to be possible based on these data alone. This research offers an approach to comprehensively identify DBPs of organic molecules and fills in much needed data on the formation of specific DBPs from three environmentally relevant sulfonamide antibiotics.

1. Introduction

Sulfonamide antibiotics (SAs) comprise a group of dozens of commercial pharmaceutical chemicals, several of which are widely known to occur as anthropogenic pollutants in water resources around the world (Garcia-Galan et al., 2011; Lindsey et al., 2001). Their occurrence in natural water systems is explained by their wide use in human and veterinary medicine (Boxall et al., 2003; Hirsch et al., 1998), their poor elimination during wastewater treatment (Gobel et al., 2005; Karthikeyan and Meyer, 2006), and their persistence in natural waters (Boreen et al., 2004; Trovo et al., 2009). Screening methods rarely consider the full breadth of SAs that may occur in water resources, but sulfamethoxazole (SMX), sulfathiazole (STZ), and sulfadimethoxine (SDM) have each been measured in drinking water resources and in finished drinking water following conventional treatment and disinfection (Benotti et al., 2009; Gros et al., 2012; Kleywegt et al., 2011; Qiao et al., 2011; Stackelberg et al., 2007; Vulliet et al., 2011).

Environmental exposure to SAs has been shown to alter the composition of bacterial communities (Kor-Bicakci et al., 2016) and may likewise induce antibacterial resistance (Baquero et al., 2008). Human exposure to SAs is most likely to occur through drinking water where the reported concentrations of SAs have been in the 1–10 ng/L range (Benner et al., 2013). This concentration range is generally considered to be too low to raise human health concerns, though synergistic effects when co-occurring with other trace pharmaceuticals and bioactive chemicals cannot be ruled out (Pomati et al., 2006). An additional concern is the transformation of pharmaceuticals (including SAs) during drinking water production, particularly in oxidation or disinfection processes, where a variety of transformation products may be formed that may be more toxic than the parent chemical (Duirk et al., 2011; Prasse et al., 2012).

Free chlorine (*i.e.*, hypochlorous acid and the hypochlorite

* Corresponding author.
E-mail address: damian.helbling@cornell.edu (D.E. Helbling).

http://dx.doi.org/10.1016/j.watres.2016.06.042
0043-1354/

anion) is a non-selective oxidant that is commonly used as a disinfectant during drinking water production. Free chlorine is widely known to react with organic matter to produce a range of disinfection byproducts (DBPs) with negative human health effects (Nieuwenhuijsen et al., 2000). Free chlorine also reacts with anthropogenic organic chemicals during disinfection processes to generate a host of DBPs with unknown or under-explored chemical structures and effects. An excellent review article was recently published that summarized the findings of 25 studies aimed at elucidating the structures of DBPs formed from pharmaceutical compounds reacting with free chlorine (Postigo and Richardson, 2014). The data confirm that free chlorine reacts with many pharmaceutical compounds, particularly those containing reduced nitrogen or sulfur functional groups or activated aromatic systems, to generate a large number of DBPs (Postigo and Richardson, 2014). More specifically, free chlorine has been shown to rapidly react with a number of SAs with second-order rate constants on the order of 10^3 to 10^4 $M^{-1}s^{-1}$ at a pH of 7.6 (Chamberlain and Adams, 2006). The structures of eight DBPs of SMX have been previously proposed (Dodd and Huang, 2004; Gao et al., 2014). The toxicity of the proposed SMX DBPs has not been studied, but it is expected that DBPs of SAs containing a conserved sulfonamide functional group or a substituted halogen may have toxic effects (Dodd and Huang, 2004; Majewsky et al., 2014).

The growing amount of data on the structures of DBPs formed in reactions between anthropogenic organic chemicals and free chlorine is exciting and opens the door to the possibility of predicting the structures of DBPs based on the structure of the parent chemical (Chen et al., 2015). Prediction of DBP structures can help prioritize DBP research and focus expensive analyses on chemicals that are predicted to generate particularly toxic DBPs. There are two main limitations that are preventing the development of predictive models for DBPs forming from free chlorination of anthropogenic organic chemicals. First, not enough data is available on the DBPs formed from structurally similar chemicals to directly assess whether structurally similar chemicals react with free chlorine in analogous ways. Second, studies aimed at elucidating DBPs of anthropogenic chemicals rarely employ emerging analytical techniques that can comprehensively identify candidate DBPs in a non-targeted way (Gervais et al., 2011; Gulde et al., 2016; Helbling et al., 2010; Negreira et al., 2015). As a result, the full spectrum of DBPs that may form in reactions with free chlorine is mostly unknown which limits our understanding of possible free chlorination reactions.

The objectives of this research were to: (i) adapt a non-target approach to comprehensively identify the structures of DBPs for a set of structurally similar compounds; and (ii) compare the putative reaction pathways to determine whether the spectrum of DBPs is analogous among the structurally similar compounds. We selected three SAs based on their known occurrence in water resources and in finished drinking water (SMX, STZ, and SDM) and conducted free chlorination experiments in batch reactors. We adapted a non-target analytical and data-processing approach that couples high-resolution mass spectrometry experiments with a differential analysis workflow to identify all products that are forming in the chlorination experiments and are detectable in the analytical window. We then mapped the DBPs to identify putative reaction pathways that are common to the three SAs or unique to an individual SA. The adaption of the non-target method and the observed pathways are important steps towards generalizing and predicting DBP formation from anthropogenic chemicals.

2. Materials and methods

2.1. Standards and reagents

Sulfamethoxazole (99.9%) was obtained from Sigma-Aldrich (Rockville, MD) and sulfathiazole (99.9%) and sulfadimethoxine (99.9%) were obtained from Fluka (Pittsburgh, PA). The structures and physicochemical properties of the SAs are provided in Table S1 of the Supplementary Data. *N*-chloro-*p*-benzo-quinoneimine (95%) was obtained from Tokyo Chemical Industry Company, Ltd. (Tamil Nadu, India); 4-aminophenol (99.9%), 3-amino-5-methylisoxazole (97%), sulfanilic acid (99.9%), aminothiazole (97%), and 5-chloro-1,3-thiazol-2-amine (95%) were obtained from Sigma-Aldrich (Rockville, MD); and nitrosobenzene (98.1%) was obtained from Fluka (Pittsburgh, PA). Stock solutions of each chemical were prepared at a concentration of 1 g/L using 100% HPLC-grade methanol and stored at −20 °C until use. A stock solution of 0.02 M potassium phosphate buffer (pH = 7.6) was prepared by combining 86.6 mL 0.2 M K_2HPO_4 and 13.4 mL 0.2 M KH_2PO_4 solutions with 900 mL nanopure water. K_2HPO_4 and KH_2PO_4 were obtained from Fisher Scientific (Pittsburgh, PA). Aqueous sodium hypochlorite solution (5% free available chlorine, FAC) was obtained from Acros Organics and was diluted to yield 50 and 500 mg/L stock FAC reagent solutions. All FAC reagents were freshly prepared before each experiment.

2.2. Experimental procedures

Free chlorination experiments for each sulfonamide antibiotic were carried out in 10 mL clear glass reactors (Fisher Scientific, Pittsburgh, PA) in triplicate. Stock solutions of each sulfonamide antibiotic, phosphate buffer (pH = 7.6), and FAC were combined to attain an initial sulfonamide antibiotic concentration of 10 mg/L and initial FAC concentrations of 0, 2, 4, 8, 16, or 48 mg/L. The relatively high concentration of sulfonamide antibiotics was selected to ensure detection of minor DBPs with the analytical methods. The range of FAC concentrations was selected to ensure conditions of under- and over-chlorination of the sulfonamide antibiotics (conditions where the parent sulfonamide is incompletely or completely transformed, respectively) to replicate stoichiometric scenarios expected to be encountered during drinking water production. The pKa of hypochlorous acid is 7.6, so phosphate buffer was used to ensure stable and equivalent amounts of the protonated and un-protonated forms as previously described (Chamberlain and Adams, 2006). The triplicate experiments conducted at 0 mg/L FAC were designated as the control group and the remaining triplicate experiments were designated as five experimental groups. The experiments were initiated by adding FAC to reactors already containing the sulfonamide antibiotic and the phosphate buffer. Each reaction was allowed to proceed to completion (approximately 10 min; time when the change in residual FAC was zero) and no quenching agent was used prior to analysis. At the end of the reaction period, samples were collected from each reactor and analyzed immediately.

2.3. Free chlorine measurements

Initial and residual free chlorine concentrations were measured by means of the *N*,*N*-diethyl-*p*-phenylenediamine (DPD) colorimetric method as previously described (American Public Health Association, 2005; Helbling and Vanbriesen, 2007). The DPD reagent reacts with free chlorine, resulting in color intensity change that is proportional to the free chlorine concentration. DPD reagents were provided by the PPD−2DPD Powder Pop Dispenser (HF Scientific, Fort Meyers, FL). Free chlorine concentrations were read