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Hydrolysis of amphenicol and macrolide antibiotics: Chloramphenicol, florfenicol, spiramycin, and tylosin

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HIGHLIGHTS

- Four antibiotics were studied: chloramphenicol, florfenicol, spiramycin, and tylosin.
- Antibiotic hydrolysis was examined under different pH and temperature regimes.
- Antibiotics persisted and exhibited no observable hydrolysis under ambient conditions.
- Acid- and base-catalyzed hydrolysis occurred at elevated temperatures (50–60 °C).
- Degradation products may remain bioactive, but less than parent compound.

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ABSTRACT

Antibiotics that enter the environment can present human and ecological health risks. An understanding of antibiotic hydrolysis rates is important for predicting their environmental persistence as biologically active contaminants. In this study, hydrolysis rates and Arrhenius constants were determined as a function of pH and temperature for two amphenicol (chloramphenicol and florfenicol) and two macrolide (spiramycin and tylosin) antibiotics. Antibiotic hydrolysis rates in pH 4–9 buffer solutions at 25 °C, 50 °C, and 60 °C were quantified, and degradation products were characterized. All of the antibiotics tested remained stable and exhibited no observable hydrolysis under ambient conditions typical of aquatic ecosystems. Acid- and base-catalyzed hydrolysis occurred at elevated temperatures (50–60 °C), and hydrolysis rates increased considerably below pH 5 and above pH 8. Hydrolysis rates also increased approximately 1.5- to 2.9-fold for each 10 °C increase in temperature. Based on the degradation product masses found, the functional groups that underwent hydrolysis were alkyl fluoride, amide, and cyclic ester (lactone) moieties; some of the resultant degradation products may remain bioactive, but to a lesser extent than the parent compounds. The results of this research demonstrate that amphenicol and macrolide antibiotics persist in aquatic systems under ambient temperature and pH conditions typical of natural waters. Thus, these antibiotics may present a risk in aquatic ecosystems depending on the concentration present.

1. Introduction

Contamination of surface and ground waters by synthetic antibiotic compounds is a potential threat to human and ecological health. As a result of increased resistance of pathogens, the use of antibiotic compounds has increased, and new antibiotics have been developed (Zinner, 2005). Approximately 3300 metric tons of antibiotics were sold in the U.S. in 2011 for use in human medicine (US FDA, 2012), and 13 000 metric tons were sold in the U.S. in 2009 for use in animal husbandry (US FDA, 2009). These large amounts have consequently led to the presence of antibiotics in environmental systems.

Releases of antibiotics to the environment are significant because a large percentage of antibiotic doses can be excreted unchanged. For example, approximately 5–10% of chloramphenicol is excreted with no metabolic transformation (Clarke et al., 1986), and 45–60% of florfenicol is excreted in urine unchanged (Liu et al.,

Abbreviations: Chlor, chloramphenicol; Flor, florfenicol; Spir, spiramycin; Tylo, tylosin; HPLC/MS/MS, high performance liquid chromatography tandem mass spectrometry; MIC, minimum inhibitory concentration.

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2003). About 94% of spiramycin is excreted in urine and feces (Chew et al., 2012), and up to 78% of tylosin doses are excreted as the parent compound (Feinman and Matheson, 1978). Furthermore, non-metabolized antibiotics excreted by humans and animals can maintain antimicrobial activity. Subbiah et al. (2011) showed that while certain antibiotics are neutralized by attachment to particles in soil–water systems (e.g., ciprofloxacin, neomycin and tetracycline), other types can remain bioactive and impact microorganisms (e.g., florfenicol and β-lactams such as ampicillin, cefoxitin and ceftiofur). Antibiotic contamination in cattle feedlots can drive selection for antibiotic-resistance in *Escherichia coli* (Subbiah et al., 2012), and antibiotic contamination of surface water has been shown to impart sub-lethal effects to aquatic biota (Park and Choi, 2008).

Antibiotic contamination of surface and ground water can occur from the introduction of municipal and agricultural waste streams into the environment. These pharmaceuticals have been found to pass through treatment systems either as parent compounds or bioactive degradation products (Mitchell et al., 2013). Antibiotic concentrations have been determined in municipal effluent receiving streams (Massey et al., 2010) and in soil leachate from contaminated manure, biosolids, and reclaimed water land application (Hamscher et al., 2005; Kinney et al., 2006; Dolliver and Gupta, 2008). Many antibiotics have been quantified at trace levels in the environment, usually at concentrations <4 $\mu g\ L^{-1}$ in water and <1 $mg\ kg^{-1}$ in sediment (Campagnolo et al., 2002; Christian et al., 2003; Kolpin et al., 2004; Massey et al., 2010). Trace levels of antibiotics have been shown to have biotic effects on aquatic organisms (González-Pleiter et al., 2013), and concerns have been raised about the impacts imparted by chronic exposure and antibiotic mixtures for which limited information exists (Kümmerer, 2009).

Hydrolysis can be a significant degradation pathway in the environment for some organic compounds, including some antibiotics (Mitchell et al., 2014). Hydrolysis of functional groups, such as esters, amides and alkyl halides, produces a less bioaccumulative compound than the parent compound because the products are more polar, resulting in higher water solubility and lower octanol water partition coefficients (Lin et al., 2007). In general, esters are more reactive than amides. Hydrolysis rates of alkyl halides vary according to the type of halide, length of the alkyl chain, and molecular steric effects (Larson and Weber, 1994). Loftin et al. (2008) showed that a lincosamide and three sulfonamide antibiotics were more stable than the macrolide antibiotic tylosin, and tylosin was more stable than three tetracycline antibiotics. β-Lactam antibiotics may be some of the more labile antibiotic compounds since they have several hydrolytically reactive moieties (Mitchell et al., 2014). Overall, there is a broad range of hydrolysis rates for antibiotic compounds.

Hydrolysis rates are a function of environmental conditions, with temperature and pH the most important parameters. For example, hydrolysis rates typically increase as temperature increases, and base-catalyzed hydrolysis predominates over acid-catalyzed and neutral hydrolysis for many organic compounds (Mabey and Mill, 1978); although water and hydroxide anion are both attracted to electron-deficient atoms, hydroxide anion is a stronger nucleophile than water. Alternatively, hydrolysis may be catalyzed in acidic solutions when protons shift the electron density of a molecule, so that a susceptible site can be attacked by water (Larson and Weber, 1994). Hydrolysis rates are also affected by ionic strength as well as the presence of divalent metal ions, oxide surfaces, and clays (Mabey and Mill, 1978; Larson and Weber, 1994; Torrents and Stone, 1994; Pusino et al., 1996). Hydrolysis of labile functional groups is a major degradation pathway in systems without a large microorganism population, such as streams, rivers, and ground waters; however, biodegradation pathways are usually predominant in wastewater.

An understanding of hydrolysis rates of antibiotics is important for predicting their environmental persistence, especially as new antibiotics are developed and released to the environment. Antibiotic hydrolysis rates and degradation product characterization, using high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS), in water with pH 4–9 has not been studied for commonly used amphenicol and macrolide antibiotics. Two commonly used and representative antibiotics from each of two classes, amphenicol and macrolide, were chosen for hydrolysis studies. The two amphenicol antibiotics have similar structures containing amide moieties, but chloramphenicol has a nitro group, whereas florfenicol has a fluorine and a sulfonyl group. These differences may potentially alter electron densities within the molecules and affect hydrolysis rates. The two commonly used and representative macrolide antibiotics, spiramycin and tylosin, were selected to evaluate the potential hydrolysis at ether linkages within the molecules. Chloramphenicol has been found in surface waters at concentrations ranging from 10 $ng\ L^{-1}$ to 19 $\mu g\ L^{-1}$ (Hirsch et al., 1999; Alder et al., 2001; Liu et al., 2009), and florfenicol has been detected in surface waters at 120–2800 $ng\ L^{-1}$ (Pouliquen et al., 2009; Wei et al., 2012). Spiramycin levels of 18–74 $ng\ L^{-1}$ have been measured in surface waters in Italy (Zuccato et al., 2005, 2010). Tylosin surface water levels around the world have been reported ranging from 2.8 $ng\ L^{-1}$ to 280 $ng\ L^{-1}$ (Kolpin et al., 2002; Calamari et al., 2003; Kim and Carlson, 2007; Watkinson et al., 2009). The objectives of this research were to (1) determine second-order acid and base hydrolysis rate constants for chloramphenicol, florfenicol, spiramycin, and tylosin over a range of temperature and pH regimes, (2) calculate Arrhenius coefficients for hydrolysis rates of the antibiotics, and (3) display hydrolysis degradation product masses and proposed structures.

2. Material and methods

2.1. Chemicals and materials

The four antibiotics, chloramphenicol, florfenicol, spiramycin, and tylosin, were analytical grade and purchased from Sigma–Aldrich (St. Louis, MO, USA). All four of the compounds have one or more hydrolytically reactive moieties. Chloramphenicol contains a secondary amide and one chlorinated alkyl chain. Florfenicol contains a secondary amide and one chlorinated and one fluorinated alkyl chain. Spiramycin and tylosin contain a lactone moiety (i.e., cyclic ester). The physical properties of the four antibiotics are listed in Table S1 (Appendix A: Supplementary data).

Methanol, sodium acetate, sodium borate, boric acid, and formic acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). Acetic acid was obtained from Fisher Scientific (Hampton, NH, USA). Double-deionized water was purified to >18 MΩ cm using a Barnstead E-pure system (Dubuque, IA, USA).

2.2. Experimental conditions

Acetate and borate buffers (10 mM) at pH 4–9 at one-pH intervals were prepared daily. These buffers are commonly used in hydrolysis studies to minimize effects caused by nucleophilic salts that can promote acid- or base-catalyzed hydrolysis (Mabey and Mill, 1978).

Reactions were conducted in 20-mL glass volatile organic analysis (VOA) vials that contained 5 mL of buffered solutions adjusted to pH 4, 5, 6, 7, 8, or 9. The solutions were incubated at 25 °C, 50 °C, and 60 °C for at least 6 h prior to antibiotic addition; a 25 μL aliquot of the antibiotic stock solution (1 $g\ L^{-1}$ in methanol) was then added to the incubated buffer and mixed for a final antibiotic

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