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Transformation of cefazolin during chlorination process: Products, mechanism and genotoxicity assessment

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HIGHLIGHTS

- Base-catalyzed electrophilic substitution occurred in cefazolin chlorination.
- Oxidation of thioether in cefazolin was found in chlorination process.
- The pH conditions impacted on the occurrence of reaction types.
- Genotoxicity had an elevation after chlorination of cefazolin.
- Reaction pathways of cefazolin chlorination were replayed in surface water matrix.

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ABSTRACT

Large quantities of cephalosporins have entered into aquatic environment in recent years, posing potential adverse effect to human health and ecological safety. In this study, cefazolin, one of widely used cephalosporins, was targeted to explore its transformation behaviors in chlorination disinfection process. With the help of ultra high performance liquid chromatography and high resolution mass spectroscopy, one chlorinated product and four oxidation products were detected in cefazolin chlorination system. The corresponding transformation pathways of cefazolin were proposed. Two kinds of reactions occurred in chlorination system, one was oxidation of thioether-sulfur to sulfoxide and di-sulfoxide, and the other was base-catalyzed electrophilic substitution of alpha-H of amide by chlorine atom. The pH value determined the occurrence of reaction types, and increasing chlorine dose promoted transformation of cefazolin. More importantly, genotoxicity in SOS/umu assay had an elevation after chlorination, which might be attributed to the formation of chlorinated product and sulfoxide during chlorination process.

1. Introduction

In the past few decades, antibiotics have drawn considerable attention due to their potential to induce the growth of resistant bacteria and pose adverse effect to human health [1]. Cephalosporins are one of the most prominent classes of β-lactam antibiotics to inhibit bacteria cell wall synthesis and treat respiratory diseases of human and livestock [2]. A recent survey showed that cephalosporins accounted for approximately 50–70% of the total antibiotics use in most of countries [3]. Some researchers claimed that cephalosporins had the potential to induce resistance in bacterial strains, and multiple antibiotic resistance genes had been detected within lagoon water and ground water [4]. There was also evidence that these resistant genes could be partially passed to humans and animals via environmental exposure, leading to decreased susceptibility to antibiotics and exerting unfavorable impact on subsequent treatments [5]. Moreover, small quantities of cephalosporins can be accumulated in humans and animals through food chains, resulting in serious problems in the long run [6]. Zhang found that cefazedone and cefazolin sodium were able to interfere with the development of tissues and organs derived from embryonic mesoderm and ectoderm of zebrafish. When intake dose was 100 μg/mL, the teratogenic rates of zebrafish caused by cefazedone and cefazolin sodium were above 50% and 97%, respectively [7].

It was reported that, except that a small percentage of cephalosporins were metabolized, a large quantity of these antibiotics were excreted from hosts and discharged into sewage as un-metabolized form. Removal of cephalosporins by conventional treatment processes in sewage treatment plants (STPs) is often incomplete [8]. Therefore, cephalosporins have been

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frequently found in multiple environmental matrices. Cefotaxim was the predominant antibiotic in the influent of Shenzhen wastewater treatment plant (China) with a concentration about 1100 μg/L [9]. A mass load study in southern China showed that cephalexin ranged from 429 to 2910 μg/day/person in influents and 88–2820 μg/day/person in effluents, which were three times greater than those in Brisbane, Australia [10]. Residual level of cefazolin in influent and effluent of wastewater treatment plants in Taiwan was 0.08–8.79 μg/L and 2.08–3.81 μg/L, respectively [11].

Disinfection is a necessary process to reduce pathogenetic risk in water treatment and wastewater reclamation [12]. Among all the disinfectants, free available chlorine (namely as FAC), including species HOCl and OCl^-, is the most used one owing to its relatively high efficiency and low cost [13]. Meanwhile, chlorine is a strong oxidant which can react with various kinds of environmental pollutants. There were reports that FAC was able to transform many kinds of antibiotics such as fluoroquinolones, tetracyclines and macrolides [14–16]. Up to now, transformation behaviors of some cephalosporins such as cefadroxil in treatment with chlorine dioxide have been studied [17]. Cefazolin, a representative for the first generation cephalosporins, has relatively high residue level in environmental media, and exhibits some adverse effects to aquatic organisms. The transformation mechanism of cefazolin during chlorination process should be of concerns.

Therefore, the objectives of this study are to comprehensively explore reaction mechanisms of cefazolin with FAC, evaluate influence of operating conditions such as pH and FAC dose on chlorination process, and screen genotoxicity of reaction system before and after chlorination. These results will throw more light on the environmental fate and potential ecological risks of cefazolin, which may contribute to optimizing chlorination disinfection process widely used in drinking water treatment.

2. Materials and methods

2.1. Reagents and solutions preparation

Cefazolin sodium with purity >97% was purchased from TCI (Japan). Structure of cefazolin is shown in Table 1. NaClO (8%) aqueous solution was obtained from Wako Co. (Japan). Formic acid (for HPLC analysis) was purchased from Acros Organics (USA). All other chemicals were of reagent grade and used without further purification. Ultrapure water gained from Millipore Purification System (Milli-Q water) was used throughout analytical experiments. All stock solutions were prepared and diluted with Milli-Q water without adding any organic co-solvent. Concentration of FAC stock solution was standardized by iodometric titration method according to the recommended protocol [18]. Agents for preparing culture medium included penicillin G sodium, D-glucose, o-nitrophenyl-β-D-galactopyranoside (ONPG), HEPES free acid, tryptone bacto. Penicillin G sodium was purchased from Inalco Spa, Milan, Italy. D-Glucose and HEPES free acid were purchased from Amresco, USA. ONPG and tryptone bacto were obtained from Becton Dickinson Co., USA.

2.2. Reaction setup

Cefazolin is aqueous soluble, its solubility in water is ca. 50 g/L. In addition, one study has found that cefazolin and other cephalosporins had not undergone measurable biodegradation in China's Xuanwu Lake after seven days [19] and had low octanol–water partitioning coefficients (log Kow: −0.13 to 1.40); thus they could not be expected to be eliminated through sorption or biotransformation in an aqueous environment. Although cephalosporin antibiotics can be strongly hydrolyzed ($t_{1/2}$ = 1–10 d at pH > 8 or < 4 at high temperatures (35–60 °C)), they are relatively stable in typical environmental waters (pH: 7, temperature: 20 °C), with very slow hydrolysis rates ($t_{1/2}$ = 18–10 d) [20,21]. In order to identify products and reveal transformation pathways of cefazolin in chlorination process, batch experiments with relatively high concentrations of cefazolin and FAC were designed. Considering the effect of pH values on species distribution and reaction activity of FAC [22], two pH conditions (pH 4.6 and 7.6) were set in experiments. Acetate buffer solution (0.2 mM, pH4.6) and phosphate buffer solution (0.2 mM, pH7.6) were used to maintain pH variance within ±0.1 unit. Chlorination experiments were performed in 25 mL borosilicate glass bottles with Teflon septa in the absence of light, under constant stirring at 25 ± 0.5 °C.

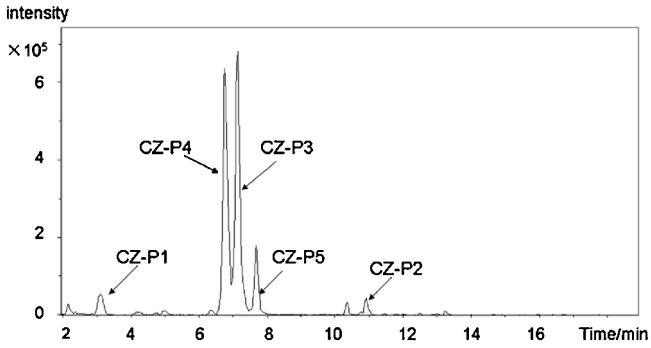

Fig. 1. Total ion chromatogram of reaction mixture after chlorination of cefazolin ($[FAC]_0$:$[cefazolin]_0$ = 3:1, 1 h, pH 7.6).

Reactions were initiated by adding a certain amount of FAC solution to 10 mL solutions containing 5 mM of cefazolin. Molar equivalent ratios of $[FAC]_0$:$[cefazolin]_0$ were set as 0, 0.1:1, 0.5:1, 1:1, 3:1, 5:1, 10:1, respectively. After 1 h, reactions were immediately quenched with a slight excessive amount of sodium thiosulfate (1.2:1 mole ratio) [23]. Aliquots after reaction were divided into two parts. The first part was centrifuged at 10,000 rpm for 10 min and analyzed by ultra high performance liquid chromatography tandem with quadruple time of flight mass spectrometer (UPLC–QTOF-MS) immediately. The other part was diluted for genotoxicity tests. All the experiments were conducted in triplicates.

2.3. Genotoxicity test procedure

To screen the potential risk of cefazolin during chlorination process, a SOS/umu assay recommended by ISO was performed [24]. The test strain *Salmonella typhimurium* TA1535/pSK1002 was provided by Prof. Yoshimitsu Oda, Japan. Simplified test procedures were provided in Supplementary materials (Text S1). In this assay, negative and positive controls were only culture medium and the water solutions of 4-nitroquinoline-N-oxide (4-NQO), respectively. Dose–response curve of the sample was obtained and then converted to an equivalent 4-NQO concentration [25]. All the tests were conducted in triplicates, and the differences in the genotoxicity of the different samples were statistically significant when $p < 0.05$ according to the Holm–Sidak test. Statistical analysis was performed using software Origin 8.0.

2.4. Product identification

According to peak intensity in the TIC (Fig. 1), major transformation products in chlorination system were separated by ultra performance liquid chromatography (Ultimate 3000, Dionex, USA) system with Agilent SB-Aq column (2.1 mm × 100 mm, 1.8 μm), and analyzed with quadruple-time of flight mass Spectrometer (micrOTOF QII, Bruker, Germany). Injection volume of UPLC was