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.

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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
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 cephalaxin 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, β-glucose, o-nitrophenyld-β-o-galactopyranoside (ONPG), HEPES free acid, tryptone bacto. Penicillin G sodium was purchased from Inalco Spa, Milan, Italy. β-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 (logKow: −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 (t1/2 = 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 (t1/2 = 18–104) [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.

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–Šidák 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 x 100 mm, 1.8 µm), and analyzed with quadruple-time of flight mass Spectrometer (micrOTOF QII, Bruker, Germany). Injection volume of UPLC was
Table 1
Chemical information of cefazolin and transformation products.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Chemical formula</th>
<th>Theoretical and measured mass</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefazolin (CZ)</td>
<td>13.9</td>
<td>C_{14}H_{15}N_{4}O_{5}S_{3}</td>
<td>455.0373</td>
<td></td>
</tr>
<tr>
<td>CZ-P1</td>
<td>3.1</td>
<td>C_{14}H_{15}N_{4}O_{3}S_{3}</td>
<td>487.0271 (487.0290)</td>
<td></td>
</tr>
<tr>
<td>CZ-P2</td>
<td>10.9</td>
<td>C_{14}H_{15}N_{4}O_{4}S_{3}</td>
<td>504.9932 (504.9960)</td>
<td></td>
</tr>
<tr>
<td>[5pt] CZ-P3 (471a)</td>
<td>6.8</td>
<td>C_{14}H_{15}N_{4}O_{3}S_{3}</td>
<td>471.0322 (471.0329)</td>
<td></td>
</tr>
<tr>
<td>CZ-P4 (471b)</td>
<td>7.2</td>
<td>C_{14}H_{15}N_{4}O_{3}S_{3}</td>
<td>471.0322 (471.0328)</td>
<td></td>
</tr>
<tr>
<td>CZ-P5 (471c)</td>
<td>7.8</td>
<td>C_{14}H_{15}N_{4}O_{3}S_{3}</td>
<td>471.0322 (471.0329)</td>
<td></td>
</tr>
</tbody>
</table>

5 μL and flow rate was 0.2 mL/min. Mobile phase A was ultrapure water containing 0.25% of formic acid, and mobile phase B was pure acetonitrile. Gradient procedures were as in Table S1. The MS^1 analyses were conducted using positive mode electrospray ionization (ESI^+) with a mass scan range of 50–1000 m/z. Temperature of spray chamber and dry gas flow rate were set at 220 °C and 4 L/min, respectively.

2.5. Computational chemistry

MOPAC software (Version 6) was used as adapted by CACHE Scientific Inc. (Oxford, UK), and the computations were performed on a Lenovo computer. PM3 parameters served to optimize the stable structures (energy minimization). The program was used to obtain the optimum geometries and partial charges.

2.6. Reaction setup with surface water samples

A surface water sample (DOC = 4.2 mg/L, [NH_4 -N] = 0.15 mg/L, pH = 8.42) was taken from a reservoir in suburban Beijing, China. Water sample was filtered through 0.45 μm glass fiber membrane and spiked with 1 μM of cefazolin and 1.2 mg/L of FAC (15 equiv. to CZ). After 1 h, the sample was concentrated 400 times by rotary evaporator and filtered through 0.22 μm membrane followed by analysis with UPLC–MS. The experiments were carried out in duplicates.

3. Results and discussion

3.1. Transformation products and pathways

According to peak intensity in the TIC (Fig. 1), a total of five major transformation products were detected during chlorination process of cefazolin. All products were well separated by gradient procedures of UPLC as shown in Fig. 1. MS^1 spectra of five products were shown in Fig. S1, in which exact mass and isotopic information provided by peaks of parent ions were utilized to deduce molecular structures of transformation products. Furthermore, MS^2 spectra (Figs. S2 and S3) of two products provided more sufficient information of fragment ions for defining their molecular structures.

Considering chemical properties of FAC (HOCl/OCl^-) and cefazolin, two kinds of reactions possibly occurred during chlorination process. One main kind of reaction was oxidation, and the other was electrophilic substitution by chlorine atom. As shown in Table 1, products CZ-P3, CZ-P4 and CZ-P5 with same m/z (471.0322) implied that they were isomeric compounds. Moreover, molecular ion
(M+H) of products CZ-P3, CZ-P4 and CZ-P5 was 16 Da larger than that of cefazolin (m/z 455.0373), indicating that one oxygen atom was added to cefazolin molecule. On basis of organic chemistry principle, sulfur atom in thioether molecule was susceptible to combine with an oxygen atom to yield sulfoxide group due to the relatively high electron density [26]. There were three sulfur atoms (designated as S1–S3 in Table 1) which were potential positions attacked by oxygen atoms.

In order to distinguish three oxidation products with m/z 471.0322, a typical oxidation system with hydrogen peroxide was selected for model study. Two oxidation products (m/z 471.0322) in hydrogen peroxide system had same retention time and mass spectra as those of 471a and 471b in chlorination system. When a low dose (8 equiv) of H2O2 was added to reaction system, only product 471b appeared. When dose of H2O2 increased (16 equiv), both 471a and 471b were found (Fig. S4). This phenomenon demonstrated that 471b was readily produced prior to 471a. As mentioned above, ease of formation of sulfoxide group had a close relation with electron density of sulfur atom, and the electrostatic potential charge is a parameter to represent electron density. So the electrostatic potential charges of sulfur atoms in cefazolin were calculated (Fig. S5). According to computational chemistry results, S1 atom had the most negative electrostatic potential charge and was more readily to be attacked by oxygen atom than S2 atom. Consequently, the proposed molecular structure of 471a and 471b were shown in Table 1. MS² spectrum of 471b using collision energy of 15 eV contained three major peaks with m/z 471.0328, 339.0496 and 321.0411. The fragmentation pathways of 471b (Fig. S2) was in line with the proposed molecular structure. Compared to S1 and S2 atoms, it was a little difficult for S3 to form sulfoxide because of the π-π conjugated effect in the five-member ring system containing S3 atom. The electrostatic potential charge of S3 atom was computed as 0.213, evidencing the relatively lower reactivity of S3 atom than that of S1 and S2 atoms.

Product CZ-P1 (m/z 487.0290) contained two more oxygen atoms than cefazolin. On basis of organic chemistry principle, when sulfur atom was attacked by oxygen atom to yield sulfoxide group, the shared electron pair between sulfur atom and oxygen atom was pulled closer to oxygen atom due to its higher electronegativity. Accompanying with the steric effect, it would become much difficult for the sulfur atom in sulfoxide group to be re-attacked by the second oxygen atom to form sulfone. This point was confirmed by the report of Spry that penicillin could not be oxidized to penicillin sulfone even with ozone, a disinfectant with higher reactivity than FAC [26]. Computational chemistry provides theoretical evidence to support the speculation as well. As shown in Fig. S5a, electrostatic potential charges of three sulfur atoms in CZ were −0.255, −0.230 and 0.213, respectively. However, when the first oxygen atom was added to S1 atom, electrostatic potential charge of S1 atom turned into 0.562 (Fig. S5b). Hence, it is not strange that the second oxygen atom would be more likely to attack S2 rather than S1 atom. MS² spectrum of product CZ-P1 and its fragment pathway (Fig. S3) also supported the proposed structure. Based on what was discussed above, the structure of product (m/z 487.0290) should be a di-sulfoxide deriving from the oxidation of S1 and S2 atoms as shown in Table 1. All these oxidation products agreed well with transformation characteristics of β-lactams in ozonation system reported by Dodd et al. [8]. The formation mechanism of sulfoxide is shown in Scheme 1. Sulfur atom in cefazolin molecule, a reactive nucloephile, undergoes electrophilic chlorination to form a chloro-sulphonium intermediate followed by hydrolysis to yield sulfoxide product [27,28].

In addition, a chlorinated product CZ-P2 (m/z 504.9960) was detected in chlorination system of cefazolin as well (Table 1). According to exact mass and isotope analysis determined by UPLC–QTOF-MS, the most possible elemental composition of CZ-P2 was C14H15N8O5S3Cl. Because of the strong electron withdrawing ability of carboxyl, neighboring alpha carbon atom had partial negative charge, so it was readily deprotonated and attacked by chlorine atom (Cl⁺) via a base-catalyzed electrophilic substitution [22]. Especially under alkaline condition, chlorine atom (Cl⁺) was more easily bound to the carbon atom to form chlorinated product. The mechanism for formation of product CZ-P2 was proposed as shown in Scheme 2. This deduction was consistent with the results of computational chemistry that electrostatic potential charge of this carbon atom was −1.30. Based on the above discussion, the proposed transformation pathways of cefazolin in chlorination process were depicted in Fig. 2.

3.2. Influence of pH

Batch experiments were conducted under acidic (pH 4.6) and neutral (pH 7.6) conditions, respectively. It was found that all five products were detected under two pH conditions except for products CZ-P2 and CZ-P5 which were absent under pH 4.6. As mentioned above, formation of chlorinated product CZ-P2 was base-catalyzed. In order to confirm it, additional chlorination experiments under pH 9.0 were also conducted. Product CZ-P2 (m/z 504.9960) was found as expected (Fig. S6). Moreover, for the same initial concentration of cefazolin (5 mM), the peak area of CZ-P2 in reaction mixture under alkaline condition was much higher than that under neutral condition. These experiment results were in agreement with the proposed formation mechanism of product CZ-P2 very well (Scheme 2). Since OH⁻ played an important role in base-catalyzed electrophilic substitution, it was reasonable that product CZ-P2 was not detected in acidic chlorination system. Product CZ-P5 has not been found in acidic chlorination system as well, the possible reason was that formation of product CZ-P5 was too scarce to be detected by instrument at pH 4.6. In sum, pH conditions had an influence on occurrence of reaction types. Base-catalyzed electrophilic substitution hardly took place under acidic pH condition.

3.3. Influence of FAC dose

Since all five transformation products were detected in neutral condition, chlorination system at pH 7.6 was taken as an example to explore the influence of FAC dose on transformation characteristics. Fig. 3 depicts the FAC-dependent relative contents of cefazolin and transformation products. It can be seen that parent CZ kept a decreasing tendency with the increasing FAC dose. Products CZ-P1 and CZ-P2 had similar pattern that they had no obvious increase until at 3 equiv. of FAC. Sulfoxide products CZ-P3 and CZ-P4 reached the first climax at 0.1 equiv. of FAC and reached the second climax at 5 equiv. of FAC. Interestingly, there was a slight decline of product CZ-P4 at 0.5 equiv. of FAC compared to that at 0.1 equiv. of FAC. This phenomenon may be attributed to the formation of products CZ-P1 and CZ-P2 which were subsequently derived from product CZ-P4. This result was in well agreement with the transformation pathways (Fig. 2) we proposed. Product CZ-P3 showed similar tendency as CZ-P4 although it did not generate products CZ-P1 and CZ-P2. The possible reason was that product CZ-P3 underwent some other transformation pathways and formed some unidentified products. Product CZ-P5 constantly accumulated until 5 equiv. of FAC. Products CZ-P3, CZ-P4, and CZ-P5 declined significantly when increasing dose of FAC to 10 equiv. Totally, judged from total ion chromatogram of reaction mixture, CZ-P1 and CZ-P2 were minor products (Fig. 1), hence the increase of products CZ-P1 and CZ-P2 were not able to balance the decrease of major products CZ-P3 and CZ-P4. This suggested that there were some other
products which were unable to be detected with present instrumental analysis method.

As for the case at pH 4.6 (Fig. S7), transformation tendency of CZ and CZ-P1 was similar as those at pH 7.6. Products CZ-P3 and CZ-P4 kept ascending and reached the peak at 3 equiv. of FAC. However, compared to the case at pH 7.6, there was no decline at 0.5 equiv. of FAC. This phenomenon was supported by the proposed transformation mechanism as shown in Scheme 1, that is, chlorinated product CZ-P2 was absent at pH 4.6. In conclusion, although different FAC dose did not change transformation pathways, it could promote the transformation of cefazolin thoroughly.

3.4. Genotoxicity results during chlorination process

It can be seen from Fig. 4 that genotoxicity after chlorination of cefazolin with different doses of FAC under pH 4.6 was lower than that under pH 7.6. This was possibly by reason of the presence of chlorinated product under pH 7.6 while absence under pH 4.6. Many previous reports showed that chlorinated organic compounds had higher genotoxicity than their parent compounds [29]. Therefore, it may be concluded that product CZ-P2 had a
certain contribution to the genotoxicity in cefazolin chlorination system.

Take the case at pH 7.6 as an example, with the increasing dose of FAC, genotoxicity had an obvious increase at 0.1 equiv. of FAC and a subsequent decrease. Similarly, the amount of sulfoxide CZ-P3 and CZ-P4 had a sudden increase at 0.1 equiv. of FAC (Fig. 3). However, genotoxicity decreased significantly at 5 equiv. of FAC even though amount of these two products (CZ-P3 and CZ-P4) reached the peak. This interesting phenomenon indicated that genotoxicity of chlorination system may be not only dependent on sulfoxide products CZ-P3 and CZ-P4 but also on cefazolin itself. S-methyl cysteine sulfide, having similar group as 471a and 471b products, showed genotoxicity on mice by inhibiting the induction of mouse micronucleated polychromatic erythrocytes [30]. As shown in Fig. 4, cefazolin exhibited obvious genotoxicity effect before chlorination treatment (at 0 equiv. FAC). On one hand, cefazolin is one of cephalosporins, which disrupts the synthesis of bacterial cell walls, thereby preventing bacterial growth. On the other hand, there are less genotoxicity (ecological toxicity) data for cefazolin in previous reports. The dose–response curve of cefazolin was prepared for SOS/umu test, which indicated that cefazolin had some genotoxicity (the data was not shown). In addition, some other in vivo and in vitro studies also proved that cephalosporins had teratogenic effects, clastogenic and aneugenic effect, cyogenetic effect [7,31–33], which indirectly evidenced the genotoxicity effect of cefazolin due to their similar molecular skeleton and functional groups.

3.5. Environmental significance

In order not to miss out any transformation products with low yields, experiments with a relatively high concentration of cefazolin (5 mM) and FAC were designed to focus on transformation characteristics. However, the experimental concentrations were much higher than the practical level of cefazolin in ambient water and FAC dosage in water treatment process. Consequently, with the object of simulating chlorination behaviors of cefazolin in drinking water treatment, experiments with low concentration of cefazolin and FAC were also conducted in surface water matrix.

The result showed that all transformation products except for product CZ-P1 were detected in the surface water matrix (Fig. S8). As mentioned above, di-sulfoxide CZ-P1 was derived from sulfoxide product CZ-P4. The absence of CZ-P1 might be in respect that many other micropolutants in surface water matrix could rival FAC with cefazolin. Hence, it was hard for sulfoxide product CZ-P4 to undergo further oxidation and form product CZ-P1. More importantly, chlorinated product and sulfoxide products with high genotoxicity were detected in surface water matrix. These results suggested that transformation of cefazolin in surface water matrix also followed the pathways we proposed, and highly genotoxic products could also be formed during chlorination process in surface water matrix. This study gave a hint that biological consequences of chlorination treatment on drinking water containing cephalosporins antibiotics should be paid attention. It is expected that the research results could be helpful for optimizing drinking water disinfection process.

4. Conclusions

Two kinds of reactions occurred during chlorination of cefazolin. One was oxidation of sulfur atoms in cefazolin, and the other was base-catalyzed chlorination substitution on the carbon atom which is adjacent to the carbonyl group. Formation of chlorinated product and sulfoxide products in chlorination system of cefazolin caused the increase of genotoxicity. The proposed transformation pathways of cefazolin chlorination could be replayed in surface water matrix, demonstrating that environmental fate and potential ecological risk of cephalosporins antibiotics deserved serious consideration.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2013.08.029.

References
