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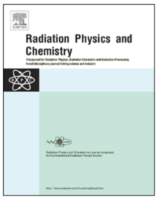

$^{\bullet}$OH and e^{-}_{aq} are yet good candidates for demolishing the β-lactam system of a penicillin eliminating the antimicrobial activity

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HIGHLIGHTS

- The aromatic side chain and the thioether moiety are the main targets for $^{\bullet}$OH.
- $^{\bullet}$OH induced deactivation of the pharmacophore occurs with ~55% efficiency.
- The carbonyl carbons are suggested to accommodate the hydrated electrons.
- Hydrated electrons destroy the antimicrobial activity with ~88% efficiency.
- Amoxicillin is demonstrated to be susceptible for one electron reduction/oxidation.

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ABSTRACT

Tracking the pharmacophore of a drug subjected to advanced oxidation is essential for evaluating the efficiency of the process in terms of wastewater treatment. From this standpoint, the $^{\bullet}$OH and e^{-}_{aq} induced deactivation mechanism of amoxicillin, a penicillin derivative was investigated in dilute aqueous solution using pulse- and gamma-radiolysis techniques. Based on IR measurements, $^{\bullet}$OH and e^{-}_{aq} destroys the β-lactam system of amoxicillin with ~55% and ~84% efficiency, respectively. In aerated solution the elimination of the pharmacophore was slightly impaired since the reaction pathway of the ring-opening was disturbed owing to the reactivity of O_2 and $O_2^{\bullet-}$ toward the intermediates of sulfur oxidation. The high potency of e^{-}_{aq} for β-lactam deactivation is attributed to the enhanced electron deficiency of the carbonyl carbon inside the lactam ring.

1. Introduction

Amoxicillin (AMX), as a broad spectrum antibiotic, is widely used in human and veterinary medicine. Owing to the enhanced consumption (European Centre for Disease Prevention and Control (ECDC) 2014; Hollis and Ahmed, 2013) added to the poor metabolism (Neu, 1974) AMX and other β-lactam antibiotics have been detected from the influent/effluent of municipal sewage treatment plants (Cha et al., 2006; Watkinson et al., 2007).

Urban wastewater treatment plants have been recognized as prominent sources for spreading antibiotic resistant bacteria and clinically relevant antibiotic resistance genes into the environment (Rizzo et al., 2013). The background of this phenomenon is the simultaneous presence of sublethal doses of antibiotics and high concentration of bacteria in these reservoirs. Therefore, avoiding the exposure of wastewater-borne and environmental microbiota to antibiotic residues is of high interest. Reactions initiated by aggressive radicals are recommended for the elimination of these molecules.

The degradation of AMX has been frequently investigated in radical reactions using photo-Fenton process (Trovó et al., 2011), microscale and nanoscale iron particles (Ghauch et al., 2009), photoreactions (Li et al., 2012), ozonation (Andreozzi et al., 2005), UV, UV/H_2O_2 and UV/$S_2O_8^{2-}$ process (Jung et al., 2012; He et al., 2014), pulse and γ-radiolysis techniques (Song et al., 2008). The degradation mainly occurs via hydroxyl radical ($^{\bullet}$OH) reactions, but under certain conditions (for instance VUV photolysis or

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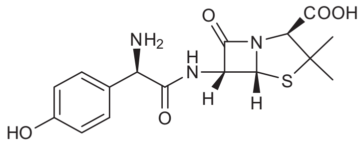
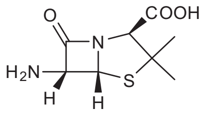
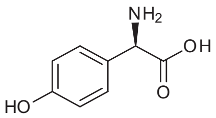


Amoxicillin
(AMX)



(+)-6-Aminopenicillanic acid
(APA)



4-Hydroxy-D-phenylglycine
(HPG)

radiolysis) other radicals, e.g. the hydrated electrons (e^-_{aq}) or the $O_2^{\bullet-}/HO_2^{\bullet}$ pair may also contribute to the reactions.

$^{\bullet}OH$ as a strong electrophile reacts with electron-rich moieties of molecules like the aromatics. In pulse radiolysis with aromatic molecules $^{\bullet}OH$ addition to the ring is well documented (Wojnárovits and Takács, 2013). The transient absorption spectrum in the $^{\bullet}OH$+AMX reaction was attributed to the absorbance of hydroxycyclohexadienyl radical (Song et al., 2008; Dail and Mezyk, 2010). The rate constant of the reaction was measured to be $(6.94 \pm 0.44) \times 10^9\ mol^{-1}\ dm^3\ s^{-1}$ (Song et al., 2008) using the absorbance build-up. Song et al. (2008) published a k_{OH} for the $^{\bullet}OH$+APA ((+)-6-aminopenicillanic acid, the antibacterial nucleus of the molecule without the aromatic ring) reaction as $(2.40 \pm 0.05) \times 10^9\ mol^{-1}\ dm^3\ s^{-1}$. The smaller value for APA than for AMX, according to their opinion "suggests that reaction of the APA moiety of the antibiotics was only a minor component of the overall $^{\bullet}OH$ oxidation". Dail and Mezyk (2010) by investigating the k_{OH}'s of seven β-lactams in pulse radiolysis concluded that "the initial hydroxyl radical reaction for all seven of the antibiotics of this study would be partitioned between the peripheral aromatic ring on the lactam substituent and the central double-ring system, with only ~30% reacting with the latter". Since the pharmacophore remains intact the inefficiency of $^{\bullet}OH$ in eliminating the antibacterial activity has also been assumed (Rickman and Mezyk, 2010).

The bicyclic system of penicillins includes a thioether moiety, an especially susceptible part of a molecule to $^{\bullet}OH$ induced oxidation. The free radical oxidation mechanism of organic sulfides is well established (Asmus, 2001), the rate constants have been reported to be diffusion-controlled (e.g. Hiller et al., 1981). Furthermore, $^{\bullet}OH$ induced one-electron oxidation of sulfur has been shown to occur readily in cyclic monosulfides (Asmus et al., 1977) and in thiaproline derivatives having the thiazolidine ring (Pogocki and Bobrowski, 2014). By resolving the transient spectra of amoxicillin, we reported a typical sulfur oxidation pathway and suggested that the $^{\bullet}OH$ attack took place at the sulfur with the same probability (Szabó et al., 2015). In end-product experiments the sulfoxide appeared with the highest abundance, however, other products reflecting e.g. ring-hydroxylation, attack at the nitrogen, oxidation of the methyl groups were also observed. Many studies have taken only the latter reactions into account (Andreozzi et al., 2005; Ghauch et al., 2009; Trovó et al., 2011; Li et al., 2012).

The hydrated electron is a very strong nucleophile, it reacts with AMX with k_{eaq^-} of $(3.47 \pm 0.07) \times 10^9\ mol^{-1}\ dm^3\ s^{-1}$, practically the same value has been found for APA $(3.35 \pm 0.06) \times 10^9\ mol^{-1}\ dm^3\ s^{-1}$. Based on the similar k_{eaq^-} for the two reactions Song et al. (2008) suggested e^-_{aq} attack on the APA part of AMX. The exact place of e^-_{aq} attack has not been reported.

In this work we investigate the free radical oxidation/reduction mechanisms that lead ultimately to β-lactam ring opening and yet elimination of the antibacterial activity.

2. Experimental

Amoxicillin (AMX), 6-aminopenicillanic acid (APA) and 4-hydroxy-D-phenylglycine (HPG) were purchased from Sigma-Aldrich. The chemicals for the analytical work provided by Spectrum-3D or Carlo Erba were of HPLC grade. A ^{60}Co facility with 11.5 kGy h^{-1} dose rate was used for γ-irradiation. AMX solutions were prepared in purified water and irradiated with varying doses under different conditions at natural pH of ~5.2. At this pH AMX is in the zwitterionic form with protonated α-amino and dissociated carboxyl group. In APA and HPG solutions the monoanionic forms dominate. The degradation of AMX in aerated solution was followed by LC/MS as described previously (Szabó et al., 2015).

Quantitative FTIR measurements were carried out using a Unicam Mattheson Research Series 1 equipment with KBr pastille method. A calibration curve was prepared by using two solutions. One of them contained 250 μg cm^{-3} antibiotic, while the other was made of 0.1 g cm^{-3} KBr and 0.2 mg cm^{-3} KSCN (internal standard). 1 cm^3 of the latter solution was admitted to the calculated amount of other solutions containing 500, 300, 150, 100, 50 and 10 μg antibiotic, and the sample was lyophilized. The powder obtained was used to prepare the pastilles. In the dose-dependence studies on $5 \times 10^{-4}\ mol\ dm^{-3}$ AMX solution, 2 cm^3 samples were handled in the same way as the solutions used for calibration. UV–vis absorption spectra were recorded on a Jasco 550 spectrophotometer using 1 cm cell.

Pulse radiolysis was conducted to observe the transient intermediates using 800 ns pulses of 4 MeV electrons, with optical detection in 1 cm cell, dose/pulse 20–40 Gy (determined using standard KSCN dosimetry) (Földiák et al., 1988).

3. Results and discussion

3.1. Spectroscopic evidence for electrophilic/nucleophilic attack on the bicyclic system

In the UV–vis spectrum of AMX two absorption bands can be distinguished (Fig. 1): the band at 272 nm is the characteristic $\pi \rightarrow \pi^*$ aromatic ring excitation band, the one with λ_{max} 228 nm is related to the amide $n \rightarrow \pi^*$ transition in the β-lactam chromophore (Boyd, 1972).

When during γ-irradiation $^{\bullet}OH$ reacts with AMX in N_2O, N_2 and aerated solutions the 272 nm UV band shifts to longer wavelengths and widens. At the same time the baseline shifts to higher absorbance and the peak at 228 nm gradually disappears (Fig. 1). The shift to longer wavelength of the $\pi \rightarrow \pi^*$ band is typical for aromatic ring-hydroxylation. Nevertheless, some of the products (penicilloic acid, sulfoxide derivatives) show similar absorbance around 270 nm, too (Gozlan et al., 2010; Szabó et al., 2015). Therefore, the longer wavelength band in irradiated samples seems to represent the overlapping spectra of several products. The decrease in the absorbance at 228 nm reflects changes on the APA part of the molecule. In N_2O- and N_2-saturated solutions (Fig. 1A and C), this band not just simply disappears but an enhanced absorbance is observable. This phenomenon is much obvious in N_2-saturated solutions (Fig. 1C), where the $^{\bullet}OH$ yield is lower (0.28 μmol J^{-1}). It indicates either the opening of the thiazolidine ring or releasing of the carboxyl group, since it would abolish the perturbation of the lone electron pair of the nitrogen in the lactam ring (Boyd, 1972) conferring higher intensity to the $n \rightarrow$