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A method for evaluating the pharmaceutical deconjugation potential in river water environments

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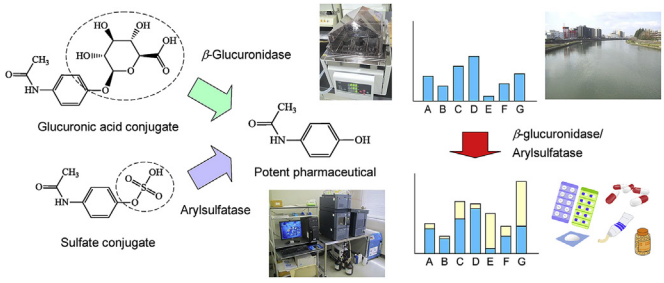
HIGHLIGHTS

- A new assay method for evaluating deconjugation activity was developed.
- Acetaminophen glucuronide and sulphate were used as model compounds.
- The reaction conditions for deconjugation were optimized.
- The method was applied to 19 pharmaceuticals in river water.
- Glucuronide conjugates predominated in the river water environment.

GRAPHICAL ABSTRACT



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ABSTRACT

A new enzymatic assay method that uses deconjugation enzymes was developed to evaluate the presence and extent of conjugated pharmaceuticals in the form of glucuronide conjugates or sulphate conjugates in river environments. First, acetaminophen glucuronide (Ace Glu) and acetaminophen sulphate (Ace Sul) were used as model conjugated pharmaceuticals to determine the appropriate combination of deconjugation enzymes and reaction conditions, including temperature, duration and pH. Next, we applied the defined method to 19 pharmaceuticals grouped into nine therapeutic classes that were chosen based on previously detected levels and frequencies in sewage and river water. The enzymatic decomposition profile varied widely depending upon the enzyme preparations available. The effect of the water reaction temperature was small between 5 and 40 °C, and the reaction proceeded in for both glucuronide conjugates and sulphate conjugates at an approximately neutral pH (corresponding to usual river water conditions) within 1 h. Application of the method to environmental samples showed that some pharmaceuticals were present in both glucuronide conjugate and sulphate conjugated forms, although glucuronide conjugates were the primary forms in the river water environment. Water treatment systems at sewage treatment plants were found to be effective for the removal of these conjugated compounds. The present results should be valuable in the environmental risk assessment of conjugated pharmaceuticals and in keeping river environments clean. To the best of our knowledge, this is the first report that enables the evaluation of the pharmaceutical deconjugation potential in a river environment.

1. Introduction

The new environmental pollution problem of pharmaceuticals in river water environments is now attracting attention worldwide

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(Vasquez et al., 2014; Evgenidou et al., 2015; Barbosa et al., 2016). The major origin of pharmaceutical environmental pollutants is their use in modern life (Petrović et al., 2013). After administration, pharmaceuticals are metabolized and then excreted; metabolism can either strengthen or weaken their pharmacological activity (Azuma et al., 2012; Funke et al., 2016). Conjugation reactions weaken pharmacological activity when in combination with glucuronic acid, sulfuric acid, or amino acids containing polar functional groups (Evans and Relling, 1999; Zheng et al., 2007; Siissalo et al., 2010). Conjugated pharmaceuticals have therefore improved polarity and water solubility and are easily excreted from the body. These reactions are not irreversible. In contrast, deconjugation reactions proceed at any place where deconjugation enzymes are present, resulting in the formation of the original pharmaceuticals (Kumar et al., 2012; Gauderat et al., 2016).

There is therefore a concern about additional pollution from conjugated pharmaceuticals in river water flows. Some researchers have already reported the development of methods for analysing conjugates and showed the occurrence of oestrogen conjugates in sewage and river waters (Liu et al., 2009; Kumar et al., 2012; Ma et al., 2016). Studies of conjugated pharmaceuticals have so far been limited primarily to antipyretic analgesic acetaminophen (Ace) (Santos et al., 2013; Azuma et al., 2016). These studies have revealed that the target conjugate compounds are distributed in river environments at the same level or sometimes at greater levels than the parent compounds (17β-estradiol (E2): 0.6–3 ng/L; 17β-estradiol 3-glucuronide (E2-3Glu): N.D.; 17β-estradiol 3-sulfate (E2-3Sul): 0.5–2 ng/L (Pedrouzo et al., 2009; Kumar et al., 2011; Zhu et al., 2015); acetaminophen: 32–250 ng/L; acetaminophen glucuronide (Ace Glu): 21 ng/L to 3.6 μg/L; and acetaminophen sulphate (Ace Sul): N.D. to 1.6 μg/L (Santos et al., 2013; Azuma et al., 2016)).

These reports suggest that further research focused on conjugate compounds in river water environments is essential to comprehend the occurrence and environmental fate of pharmaceuticals and evaluate their impact on aquatic ecosystems in detail. However, such research remains very limited (Brown and Wong, 2015). The primary reason is in that appropriate standard chemicals for qualification and quantification are not commercially available. In addition, there are practical methodological problems associated with the analysis of a wide variety of compounds having different physicochemical properties (Brown and Wong, 2016). One effective way for overcoming these problems would be the development of a new method that enables the estimation of target conjugates in the form of each mother pharmaceutical using appropriate deconjugation enzymes. The amount of the conjugates could be evaluated as an increment of each mother pharmaceutical. However, to the best of our knowledge, this type of trial research has not yet been reported.

Given this situation, the present research was focused on the estimation of glucuronide and sulphate conjugates, which account for the bulk of conjugates in humans (Williams et al., 2004; Brauch et al., 2009). Ace Glu and Ace Sul were chosen as the model conjugates and their amounts were estimated from newly formed Ace through enzyme reactions. First, the deconjugation reaction was optimized for the selection of an appropriate enzyme preparation and reaction condition with regards to temperature, pH, and incubation time duration. Second, the defined method was applied to detect 19 mother pharmaceuticals, including Ace, categorized into nine therapeutic classes in urban river water and sewage treatment plant (STP) effluent samples in Japan. Third, the data were used to evaluate pharmaceutical occurrences as conjugates in the river environment and, finally, the effectiveness of the developed method was discussed.

2. Materials and methods

2.1. Chemicals and reagents

Ace was purchased from the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Ace Glu (purity 98%) was purchased from Sigma-Aldrich LLC. (St. Louis, MO, USA) and Ace Sul (purity 98%) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). The physicochemical properties of Ace, Ace Glu, and Ace Sul are summarized in Table S1. Two types of enzyme preparations, β-glucuronidase (β-Glu) and arylsulfatase (ASul) mixed with β-Glu activity (β-Glu/ASul), were purchased from Roche Diagnostics K.K. (Basel, Schweiz), Sigma-Aldrich Co., MP Biomedicals LLC (Santa Ana, CA, USA), and Nacalai Tesque, Inc. The properties of each enzyme preparation are summarized in Table S2. Individual standard stock solutions of Ace Glu, Ace Sul, and Ace at 1 mg/100 mL were prepared in methanol and stored at −20 °C. Liquid chromatography – mass spectrometry (LC-MS) grade solvents (methanol and acetone), formic acid, hydrochloric acid, sodium hydroxide, ammonia, and ascorbic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All solutions were prepared with ultra-pure water (18.2 MΩ cm) from a Milli-Q purification system (MilliporeSigma, Watford, UK).

2.2. Enzymatic deconjugation reaction

2.2.1. Selection of enzymes

A total of 7 enzyme preparations, including three β-Glu and four binary β-Glu/ASul reactive enzymes, were obtained from multiple companies (Table S2) to evaluate the extent of deconjugation of glucuronide and sulphate. Thirty-millilitre solutions of Ace Glu or Ace Sul were prepared at 100 μg/L in MilliQ-water in separate glass bottles with screw caps and shaken at 100 rpm for 1 h in a water bath shaker (NTS 4000BH, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 40 °C in the dark after the addition of each enzyme preparation whose concentration was set to make the β-Glu activity equivalent to 1000 units.

After incubation, the reaction solutions were immediately cooled in an ice bath. Their pH was adjusted to 3 in accordance with sample pretreatment using solid phase extraction (SPE) and analysed using ultra-performance LC (UPLC)-MS/MS (section 2.3).

2.2.2. Temperature dependence of enzymatic deconjugation

The effect of incubation temperature on deconjugation was analysed at a temperature range from 5 to 40 °C which were chosen to approximate the annual temperatures of river and STP waters (Ministry of Land Infrastructure Transport and Tourism, Japan, 2017; Japan Sewage Works Association, 2016) and the temperature ranges required by living organisms (Busto et al., 1987). As described in section 2.2.1, 30-mL solutions containing 100 μg/L of Ace Glu or Ace Sul in MilliQ-water was incubated with each enzyme preparation for 10 min at 5, 10, 20, 30, and 40 °C. The concentration of all enzyme preparations was set to make the β-Glu activity equivalent to 10,000 units. After incubation, the reaction solutions operated as described in section 2.2.1.

2.2.3. Reaction time dependence of enzymatic deconjugation

The effect of the reaction time on deconjugation reactions was analysed via the incubation of 30-mL solutions containing 100 μg/L of Ace Glu or Ace Sul in MilliQ-water with all enzyme preparations separately for 0, 10, 30, 60, 120, and 180 min at 40 °C. In all cases, the amount of every enzyme preparation was set to make the β-Glu activity equivalent to 10,000 units. The reaction solutions were then analysed as described in section 2.2.1.