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Characterization of chemo- and regioselectivity in enzyme-catalyzed consecutive hydrolytic deprotection of methyl acetyl derivatives of 1-β-*O*-acyl glucuronides

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ABSTRACT

Methyl acetyl derivatives of 1-β-*O*-(*o*-, *m*-, or *p*-phenyl)benzoyl glucuronides **2a**–**c** are fully deprotected by a one-pot consecutive enzyme-catalyzed hydrolytic reaction to afford **4a**–**c**, without isolation of the *O*-deacetylated derivatives **3a**–**c**. A lipase AS Amano from *Aspergillus niger* (LAS) and a carboxylesterase from *Streptomyces rochei* (CSR) showed high chemoselectivity toward the *O*-deacetylation of the *o*- and *m*-isomers, respectively. Chemoselective *O*-deacetylation of the *p*-isomer was promoted only in the presence of both enzymes. A lipase type B from *Candida antarctica* (CALB) was effective for the subsequent enzymatic hydrolysis of the methyl esters of **3a**–**c**. LAS exhibited also regioselective 3-*O*-deacetylation activity to afford the corresponding 2,4-di-*O*-acetyl intermediates **5a**–**c**, for which CSR showed higher *O*-deacetylation activity than that for **2a**–**c**. In kinetic studies using *p*-nitrophenyl ester substrates, LAS exhibited a broader acyl preference, the octanoyl ester being most effectively hydrolysed, whereas CSR exhibited the highest hydrolytic activity toward the acetyl ester. LAS and CSR play complementary as well as synergistic roles in the *O*-deacetylation of 2 bearing R groups of different steric bulkiness.

1. Introduction

It is important to understand pathways for the metabolic activation of drugs to reactive metabolites and subsequent irreversible covalent binding to target tissue macromolecules, as these processes are implicated in adverse drug reactions. Therefore, in discovery and development of safer drugs, efficient and widely applicable synthetic methodologies are required for preparing these reactive metabolites as standard samples and materials for toxicological and pharmacokinetic studies. 1-β-*O*-Acyl glucuronides (βGAs), a group of the above-mentioned reactive metabolites, are in general electrophilic species capable of binding to tissue proteins [1–4], possibly leading to adverse reaction of the parent carboxylic acid drugs [5–7]. For example, some non-steroidal anti-inflammatory drugs have been withdrawn due to their toxicities [8–10]. However, specific target macromolecules to which βGAs could covalently bind, toxicological consequences of the covalent binding, and relationships between the electrophilicity of βGAs and the toxicity of the parent carboxylic acid drugs remain largely unknown.

We have recently established a methodology for chemoenzymatic synthesis of βGAs with exclusive β-selectivity (Scheme 1) [11–13], and derived quantitative structure–activity relationships for the electrophilicity of their 1-β-*O*-acyl linkages to predict the electrophilic reactivity [13–15].

Among the enzymes screened under the conditions shown in Scheme 1, a lipase from *A. niger* (LAS) and a carboxylesterase from *S. rochei* (CSR) showed hydrolytic activity for the deprotection of the *O*-acetyl groups of the methyl acetyl precursor **2** to the corresponding methyl ester derivative **3**, whereas a lipase type B from *C. antarctica* (CALB) and an esterase from porcine liver (PLE) showed hydrolytic activity toward the methyl ester group of **3** to afford the desired βGA **4**. So far the chemoenzymatic method has been successfully applied to synthesis of 15 kinds of βGAs derived from six aromatic and nine aralkyl carboxylic acids [11–15]. LAS and CSR showed a good chemoselectivity toward the *O*-acetyl group among the three types of the ester functions of the compound **2**: the *O*-acetyl group, the 1-β-*O*-acyl linkage, and the carboxyl methyl ester. However, the structure of R group of 1-β-*O*-acyl linkage in a given compound **2** has been proved to considerably affect the chemoselectivity as well as the catalytic activity of these enzymes. For example, toward compounds **2** with phenyl and benzyl groups as the R group, LAS did not show good chemoselectivity toward the *O*-acetyl groups and unfavorably cleaved the 1-β-*O*-acyl linkage to liberate the parent benzoic and phenylacetic acids, respectively, whereas CSR showed much higher chemoselectivity toward these substrates to afford the corresponding fully *O*-deacetylated compounds **3** in good yields [12]. Furthermore, LAS exhibited high chemoselective *O*-deacetylation activity toward **2** with a bulky *o*-(anilino)phenyl group as the R group,

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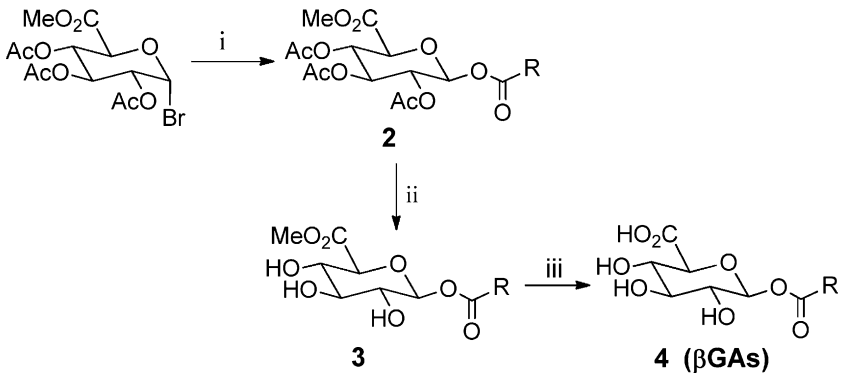
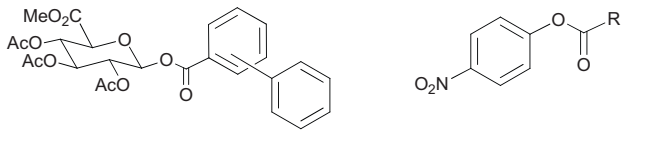


Scheme 1. Chemoenzymatic synthetic route to βGAs **4**. (i) RCO_2Cs, DMSO; (ii) LAS and/or CSR, 20% (v/v) DMSO in citrate buffer (pH 5.0); (iii) CALB, 20% (v/v) DMSO in citrate buffer (pH 5.0).

whereas CSR did not exhibit good hydrolytic activity toward this substrate [12]. In addition, only concurrent use of LAS and CSR synergistically showed good chemoselective *O*-deacetylation activities toward **2** with *p*-biphenyl and α,α-diethyl- and α,α-dimethyl-(*p*-phenyl)benzyl groups as the R group [12,13]. For these reasons, LAS and CSR are likely to play complementary and also synergistic roles in the hydrolytic deprotection of the *O*-acetyl group of **2** but the relationship between their hydrolytic activity and the structure of the R group has yet to be determined. Furthermore, LAS is different from CSR in that LAS is prone to afford some partially *O*-deacetylated intermediates in the reaction course.

There are several reviews for applications of hydrolytic enzymes to the regioselective *O*-deacylation of peracylated carbohydrates [16–19]. Among the enzymes, *A. niger* lipase has been reported to catalyze regioselective hydrolyses of anomeric *O*-acetyl esters of per-*O*-acetylated β-D-glucopyranose [20,21], β-D-ribofuranose [22] and oligosaccharides [23,24]. LAS-catalyzed chemoselective *O*-deacetylation of **2** to afford **3**, leaving the 1-β-*O*-acyl linkage intact, might strongly depend on the bulkiness of the R group of **2**. On the other hand, regioselective *O*-deacetylation activity has not been reported for CSR-catalyzed ester hydrolysis to the best of our knowledge.

It is, therefore, important and of interest to study the acyl preference and the influence of the steric effect of the R groups on LAS- and CSR-catalyzed chemo- and regioselective *O*-deacetylation of **2**, and thereby to gain insight into the enzymatic characteristics in the chemo- and regioselectivity. It has to be confirmed also the acyl preference with respect to the steric effect of R groups not yet available in the literature. Furthermore, a consecutive one-pot deprotection procedure from **2** to **4**, without isolation of **3**, seems to be an intriguing synthetic approach. Among the abovementioned 15 kinds of βGAs, the methyl acetyl derivatives of regioisomeric βGAs **2a**–**c** derived from *o*-, *m*- and *p*-phenylbenzoic acids **1a**–**c** (Scheme 2) have been selected as model substrates, because the compounds **2a**–**c** were *O*-deacetylated under different conditions using LAS, CSR, and concurrent use of LAS and CSR, respectively [12,13]. In this study, we report (1) the chemo- and regioselectivity of LAS and CSR toward **2a**–**c**, to characterize the effects of the R group, (2) acyl preferences in LAS- and CSR-catalyzed hydrolysis using several *p*-nitrophenyl esters **7a**–**e** (Scheme 2) by analysing the reaction kinetics, and (3) a one-pot deprotection procedure from **2a**–**c** to the corresponding 1-β-*O*-acyl glucuronides **4a**–**c**.



2a; *o*-isomer
2b; *m*-isomer
2c; *p*-isomer

7a; R = CH_3 **7d**; R = $(CH_2)_6CH_3$
7b; R = CH_2CH_3 **7e**; R = C_6H_5
7c; R = $(CH_2)_2CH_3$

Scheme 2. Substrates used for LAS- and CSR-catalyzed hydrolysis.

2. Experimental

2.1. General methods and materials

Lipases AS Amano from *A. niger* (LAS) and from *C. antarctica type B* (CALB), a carboxylesterase from *S. rochei* (CSR), and *o*-, *m*-, and *p*-phenylbenzoic acids were obtained from Wako Pure Chemical Industries (Osaka, Japan). Novozym 435 (an immobilized form of CALB) was purchased from MIK Pharmaceuticals Co., Ltd. (Tokyo, Japan) and Lipozyme® CALBL was kindly gifted from Novozymes Japan Ltd. (Chiba, Japan). The methyl acetyl derivatives **2a**–**c** were synthesized according to our previous papers [11–13]. *p*-Nitrophenyl esters **7a**, **7c**, and **7d** were obtained from Nacalai tesque Inc. (Kyoto, Japan); the propionyl [25] and benzoyl [26] esters **7b** and **7e** were synthesized according to reported procedures. Amberlite XAD-4 was obtained from the Organo Corporation (Tokyo, Japan) and used after grinding (80–200 mesh). All other chemicals were commercial products. ^1H and ^{13}C NMR were recorded using a JNM-AL400 (JEOL) and the chemical shifts are presented as δ-values in ppm with reference to the residual solvent signals [27] of d_6-DMSO (2.49 ppm for ^1H NMR and 39.50 ppm for ^{13}C NMR) or CD_3OD (3.30 ppm for ^1H NMR and 49.00 ppm for ^{13}C NMR). MS and HRMS were measured by electron impact (EI) ionization using a Hitachi M-2000 spectrometer.

2.1.1. General incubation conditions for enzymatic hydrolyses

The reaction mixtures were incubated at pH 5.0 to minimize a non-enzymatic intramolecular acyl migration of partially and fully *O*-deacetylated intermediates formed from **2a**–**c**. The progress of enzyme-catalyzed hydrolyses were monitored using HPLC. Unless otherwise indicated, the incubation was performed in 20 mM sodium citrate buffer (pH 5.0) containing 20% (v/v) DMSO as a co-solvent and at 40 ± 0.1 °C for LAS and CALB (including Lipozyme® CALBL and Novozym 435) and at 50 ± 0.1 °C for CSR, respectively. Among several cosolvents tested, DMSO was the best cosolvent for LAS and CSR using **2a** and **2b** as substrates, respectively. The concentration of LAS and CSR was 10 mg/mL of the incubation mixture. The incubation with both enzymes was performed at 40 ± 0.1 °C; the optimal temperature of LAS toward **2a** was 40 °C and the activity was gradually deactivated at 50 °C, whereas the catalytic efficiency of CSR was optimal at around 50 °C [12]. Incubation was started by the addition of substrate in DMSO solution to the incubation mixture. Hydrolytic activity of LAS and CSR toward **5a**–**c** was presented