Process Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

OcUGT1-catalyzed glycosylation of testosterone with alternative donor substrates

Shuai Yuan, Sen Yin, Ming Liu, Jiu-Ming He, Jian-Qiang Kong*

Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College (State Key Laboratory of Bioactive Substance and Function of Natural Medicines & Ministry of Health Key Laboratory of Biosynthesis of Natural Products), Beijing, 100050, China

ARTICLE INFO

Keywords:
Glycosylation
Glycosyltransferase
Testosterone
Transglycosylation
*o*NP-Glc

ABSTRACT

Testosterone 17-*O*-glucoside (T-17-G) is a precursor of the anticancer compound 3′-acetylated testosterone 17-*O*-β-glucoside (3′-AT-G). The biosynthesis of T-17-G was achieved by the glycosyltransferase (GT)-mediated glycosylation of testosterone with UDP-Glc. The low availability of UDP-Glc was detrimental to the synthesis of T-17-G, thereby limiting the druggability studies of 3′-AT-G. Therefore, finding cheaper alternatives of sugar donors is particularly urgent for the biosynthesis of T-17-G. Here, we reported the biosynthesis of T-17-G based on the transglucosylation reaction and an alternative sugar donor 2-nitrophenyl β-D-glucopyranoside (*o*NP-Glc) was thus determined. Specifically, a flavonoid glycosyltransferase OcUGT1 from *Ornithogalum caudatum* was used as a biocatalyst to test the reactivities of various sugar donors with testosterone. The results indicated that OcUGT1 was able to catalyze the glucosylation of testosterone with UDP-Glc, thereby forming T-17-G and a diglucoside. Also, the generation of T-17-G was achieved by OcUGT1-assisted transglucosylation between testosterone with *o*NP-Glc or 4-nitrophenyl β-D-glucopyranoside (*p*NP-Glc). Moreover, the glucosylation efficiency of testosterone in the presence of *o*NP-Glc was found to be higher than that with UDP-Glc. This data, together with the high availability of *o*NP-Glc revealed its applicability as a promising alternative to UDP-Glc in the glucosylation of testosterone.

1. Introduction

The compound 3′-acetylated testosterone 17-*O*-*β*-glucoside (3′-AT-G) is an acetylated testosterone glycoside with potent anticancer activity (Fig. 1). The biosynthesis of 3′-AT-G had been previously achieved in our laboratory [1]. There are two enzymes responsible for the biosynthesis of 3′-AT-G. The first is a steroidal glycosyltransferase (SGT) designated as OsSGT1 from *Ornithogalum saundersiae*. Under the action of OsSGT1, testosterone (**1**) was glycosylated with UDP-Glc to form testosterone 17-*O*-glycoside (T-17-G). Next, the hydroxyl groups at C-2′, -3′, -4′, and -6′ positions in the sugar moiety of T-17-G were acetylated with a steroidal glycoside acetyltransferase (SGA), thereby forming 2′, -3′, -4′, and -6′-AT-G (Fig. 1). Of these acetylated testosterone glycosides (ATG), 3′-AT-G showed extensive toxic activity towards cancer cells, suggesting promising anticancer potential [1]. Thus, for further druggability studies, the improvement of the biosynthetic yield of 3′-AT-G is urgent. However, the utilization of expensive UDP-Glc in the OsSGT1-catalyzed glycosylation limits the scale preparation of biosynthetic 3′-AT-G. Therefore, it is necessary to find alternative sugar donors for the biosynthesis of T-17-G, to sequentially improve the biosynthetic yield of 3′-AT-G for further druggability studies.

O-Aryl substituted glucosides like 2-nitrophenyl β-D-glucopyranoside (*o*NP-Glc, **2**) and 4-nitrophenyl β-D-glucopyranoside (*p*NP-Glc, **3**) were usually used as donors for transglucosylation [2–9]. Being readily available, these aryl glucosides are used to synthesize flavonoid *O*-glucosides in transglucosylation reactions [10,11]. Therefore, they could serve as alternatives to UDP-Glc for the synthesis of small molecular glucosides.

OcUGT1 is a multifunctional flavonoid glycosyltransferase from *Ornithogalum caudatum* with glycosidase activity [11]. OcUGT1 is able to catalyze the generation of new glycosides with the addition of expensive UDP-Glc. Additionally, the formation of new glycosides was achieved by OcUGT1-catalyzed transglycosylation with cheaper aryl substituted glycosides as the sugar donors [11]. This evidence suggests that testosterone(**1**) could also be glycosylated in an OcUGT1-catalyzed transglycosylation fashion. Thus, in this investigation, OcUGT1 was used as a biocatalyst to test the reactivities of diverse aryl glucosides with testosterone (**1**). The results indicated that the OcUGT1-assisted glycosylation of testosterone (**1**) was achieved with at least three sugar donors, including UDP-Glc, *o*NP-Glc, and *p*NP-Glc. Moreover, the

* Corresponding author.
E-mail address: jianqiangk@imm.ac.cn (J.-Q. Kong).

https://doi.org/10.1016/j.procbio.2018.08.005
Received 6 June 2018; Received in revised form 18 July 2018; Accepted 2 August 2018

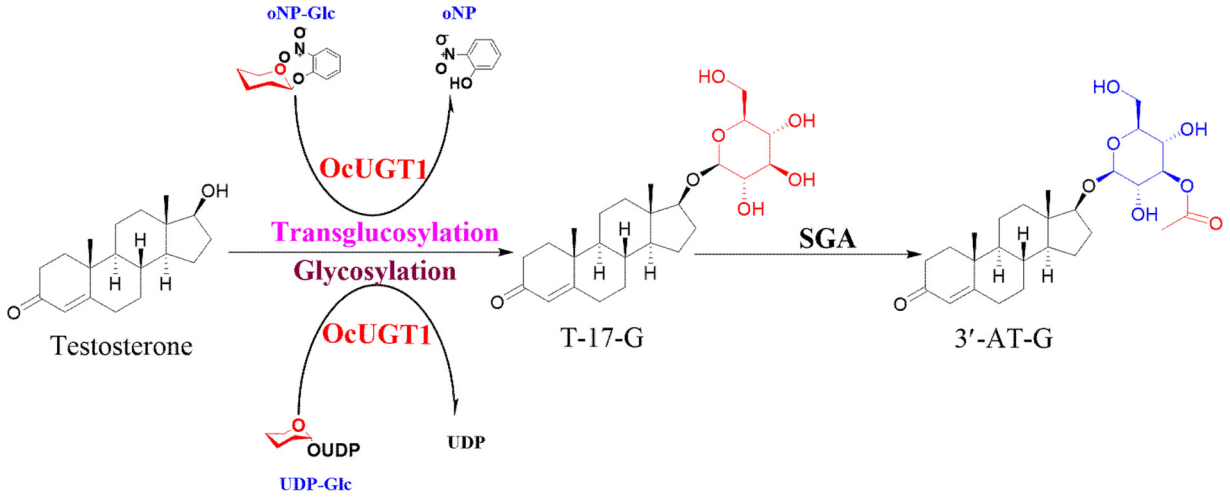

Fig. 1. The biosynthetic pathway of 3′-AT-G.

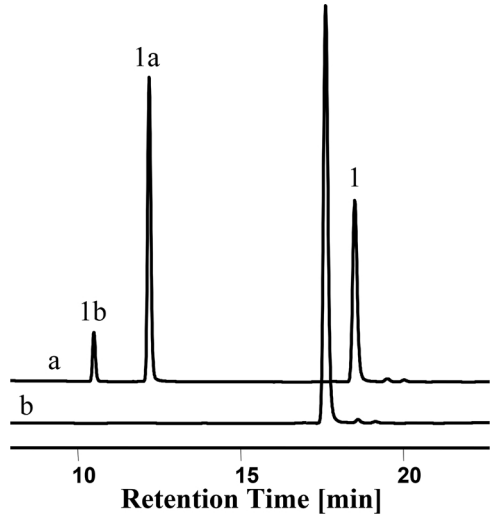

Fig. 2. HPLC profiles of glucosylated metabolites of testosterone (1) with UDP-Glc. a, HPLC chromatogram of glucosylation reaction in the presence of OcUGT1.
b, HPLC chromatogram of glucosylation reaction in the absence of OcUGT1.

conversion rate from *o*NP-Glc to T-17-G is superior to that of UDP-Glc to T-17-G, indicating that the former is a promising alternative to UDP-Glc for the formation of T-17-G. This study will lay the foundation for the druggability study of 3′-AT-G.

2. Materials and methods

2.1. Enzyme and chemicals

OcUGT1 was previously purified and used as the biocatalyst for glycosylation in this investigation [12]. UDP-Glc was purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). The glycosides listed in Figure S1 were obtained from J&K Scientific Ltd (Beijing, China). Other chemicals were of either reagent or analytical grade when available.

2.2. Glycosylation assay of testosterone

The OcUGT1-catalyzed glycosylation assay of testosterone (**1**) was performed in 100 µl of phosphate buffer (10 mM, pH 8.0) containing 10 µg purified OcUGT1, 1 mM testosterone and 1 mM UDP-Glc. The reaction mixture was incubated at 50 °C for 2 h and then quenched by adding 100 µl methanol and 10 µl acetic acid. The glycosylated products were monitored by high performance liquid chromatography (HPLC). The detailed chromatography conditions were the same as described previously [11,13,14]. ESI-HRMS and MS/MS analyses were performed on a Q-TOF mass spectrometer (AB SCIEX, Triple TOF™ 5600$^+$).

2.3. Transglycosylation assay of testosterone

The reaction mixture of the OcUGT1-catalyzed transglycosylation of testosterone (**1**) was the same as that of the OcUGT1-mediated glycosylation, except that the sugar donor UDP-Glc was changed to the alternative glycoside donors. The transglycosylation reaction was conducted at 37 °C for 2 h and then stopped by adding 100 µl methanol and 10 µl acetic acid. HPLC analysis and structural characterization of compounds was performed as mentioned above.

3. Results and discussion

3.1. OcUGT1-catalyzed glucosylation of testosterone with UDP-Glc

First of all, the glycosylating activity of OcUGT1 towards testosterone(**1**) with UDP-Glc was explored. Ten micrograms of purified OcUGT1 prepared previously was used as the biocatalyst. After incubation at 50 °C for 2 h, the reaction mixture containing the purified OcUGT1, testosterone (**1**) and UDP-Glc was subject to reverse HPLC analysis. As shown in Fig. 2, two new peaks designated as **1a** and **1b** were present in the reaction mixture. On the contrary, there were no new peaks present in the control mixture containing no OcUGT1, suggesting the newly formed peaks might be the reaction products catalyzed by OcUGT1 (Fig. 2). The quasi-molecular ion $[M+H]^+$ at *m/z* 451.2690 of compound **1a** was observed by ESI-HRMS indicating its formula as $C_{25}H_{39}O_7$, which was 162 Da greater than testosterone (**1**) and thus suggested the presence of a glucosyl residue (Figure S2). The MS/MS analysis of $[M+H]^+$ ion was further performed, as shown in Figure S3, the diagnostic product ion at *m/z* 289.2393 and the neutral loss of 162 Da was observed, indicating a glucosyl group was lost from a protonated molecular ion and corresponding to the mass of an aglycone ion. Moreover, the fragmentation pattern of the product ion at *m/z* 289.2393 was the same as that of the standard testosterone (Figure S3), further supporting the aglycone ion was testosterone (**1**) [15]. This MS data, together with co-elution with the standard T-17-G, unambiguously assigned this monoglucosylated product **1a** to be T-17-G (Figure S4). Compound **1b** showed a quasi-molecular ion $[M+H]^+$ at *m/z* 613.3230 in the positive ion mode ESI-HRMS spectrum, which was calculated as being a formula of $C_{31}H_{49}O_{12}$ (Figure S2). In the MS/MS spectrum, an *m/z* 451.3145 $[M+H\text{-Glc}]^+$ ion and an *m/z* 289.2363