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First and facile enzymatic synthesis of β-fucosyl-containing disaccharide nucleosides through β-galactosidase-catalyzed regioselective glycosylation

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ABSTRACT

β-Galactosidase from bovine liver was purified to homogeneity. Its molecular mass was estimated to be 54 kDa by SDS-PAGE, 60 kDa by gel permeation chromatography, and 57 kDa by matrix-assisted laser desorption ionization – time of flight tandem mass spectrum. This enzyme displayed the highest catalytic efficiency with *p*-nitrophenyl β-D-galactopyranoside (V_{max}/K_m value, 0.0173 min^{-1}) as the substrate, lower with *p*-nitrophenyl β-D-fucopyranoside (0.0156 min^{-1}) and the lowest with *p*-nitrophenyl β-D-glucopyranoside (0.0126 min^{-1}). With the enzymatic fucosylation of floxuridine as a model reaction, four key reaction conditions were optimized. Under the optimum conditions, the enzymatic synthesis of a group of β-fucosyl-containing disaccharide nucleosides using the purified β-galactosidase was conducted. The desirable 5′-*O*-β-D-fucosyl derivatives of pyrimidine nucleosides were obtained with 44–60% yields. Besides, the 5′-regioselectivities decreased markedly with increasing bulk of 5-substituents present in the base moiety of nucleosides. In addition, the enzyme could accept acyclic nucleoside analogs as the substrates and catalyze the enzymatic fucosylation of these nucleosides, furnishing the glycosylated products with the yields of 32–36%.

1. Introduction

Nucleoside analogs constitute a vital class of anticancer and antiviral agents (De Clercq, 2004). For example, floxuridine (5-fluoro-2′-deoxyuridine, FUdR) has been used extensively in the clinical treatment of colon carcinoma and hepatic metastases (Kemeny et al., 1999); the acyclic nucleoside analog - acyclovir is effective for the treatment of herpes virus infections (O'Brien and Campoli-Richards, 1989). However, nucleoside drugs generally suffer from low oral bioavailability due to poor intestinal absorption, thus requiring large doses and frequent administration to maintain the plasma concentrations above the threshold for effective treatment; additionally, these nucleoside analogs are poorly selective for virus-infected/tumor cells and also are toxic against normal cells, leading to strong side effects in the clinical utility (De Clercq and Field, 2006; Wiebe and Knaus, 1999). Prodrugs strategy has been established well for the improvement of therapeutic efficacy by enhancing oral absorption, selectivity and water-solubility of the parent agents, etc. (Li et al., 2008). For instance, glycosidic prodrugs of many pharmacologically active compounds displayed much higher therapeutic efficacy and/or selectivity than the parent (Egleton et al., 2001; Tietze et al., 2010). The water-solubility of the antitumor drug – geldanamycin was markedly improved upon glycosylation modification (Wu et al., 2012). Likewise, *O*-glycosylated derivatives of nucleosides (disaccharide nucleosides) were reported to significantly mask their toxicity (Abraham et al., 1994; Watanabe et al., 1981).

On the other hand, many disaccharide nucleosides occur naturally, and are of pharmaceutical interest since they manifest a broad spectrum of biological activities including antibacterial, fungicidal, antitumor and antiviral activities, etc. (Efimtseva et al., 2009). For example, a fucosyl-containing disaccharide nucleoside derivative - shimofuridin A, which was isolated from marine tunicate *Aplidium multiplicatum*, was active against gram-positive bacterium and fungus as well as murine lymphoma L1210 cells (Kobayashi et al., 1994). In addition, disaccharide nucleosides were identified as the structural elements of biopolymers (Efimtseva and Mikhailov, 2002). For example, these glycosylated derivatives of nucleosides were generally found to be located at position 64 in initiator tRNA of lower eukaryotes and some plants (Keith et al., 1993). Besides,

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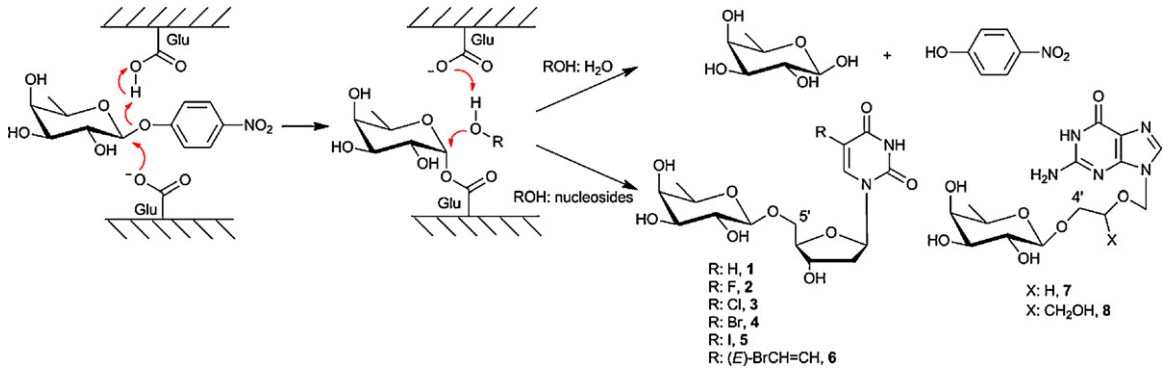

Scheme 1. Enzymatic synthesis of β-fucosyl-containing disaccharide nucleosides and possible synthetic mechanism.

these nucleoside derivatives are vital building blocks for the synthesis of oligonucleotides (Efimtseva et al., 2001), a promising class of chemotherapeutic agents.

However, the synthesis of this class of compounds remains challenging for organic chemists, owing to the presence of multi active hydroxyl and/or amino groups in the reactants. In general, arduous and tedious protection/deprotection steps and environmentally unfriendly catalysts were involved in traditional chemical methods (Efimtseva et al., 2009). Use of enzymes as the alternative to chemical catalysts offered many new opportunities for the regioselective glycosylation, because of excellent selectivity, simplicity, mild reaction conditions and being environmentally benign (Li et al., 2010). However, there are limited reports on enzymatic glycosylation of nucleosides in the two decades (Binder et al., 1995). Andreotti et al. synthesized a group of 5′-*O*-β-D-galactosyl nucleoside derivatives with the yields of 12–80% using the marine β-galactosidase from *Aplysia fasciata* (Andreotti et al., 2007). Blazek et al. reported enzymatic galactosylation of a series of acyclic nucleoside analogs using β-galactosidase from *Escherichia coli* (Blazek et al., 2012). Recently, our group has demonstrated that crude β-galactosidase from bovine liver is a promising biocatalyst for regioselective galactosylation modification of pyrimidine nucleosides (Ye et al., 2012; Zeng et al., 2010). In addition, a group of β-glucosyl-containing disaccharide nucleosides were firstly synthesized with 22–72% yields through this enzyme-catalyzed highly regioselective glucosylation (Ye et al., 2011). Encouraged by these interesting results, β-galactosidase from bovine liver was purified to homogeneity, and its biochemical properties, especially the substrate specificity, were characterized in the present work. According to its substrate specificity, we attempted to synthesize a variety of β-fucosyl-containing disaccharide nucleosides through regioselective fucosylation using this enzyme (Scheme 1). In addition, the enzyme-substrate recognition was discussed based on the rational design of the structures of nucleoside molecules. To our knowledge, this is the first report on enzymatic regioselective fucosylation of nucleosides.

2. Materials and methods

2.1. Materials

FUdR was purchased from Shanghai Hanhong Chemical Co. (China). 2′-Deoxyuridine and idoxuridine were bought from Tuoxin Biotechnology & Science (China). Acyclovir, ganciclovir and brivudine were bought from Dalian Meilun Biotech Co. (China). 5-Bromo-2′-deoxyuridine, 5-chloro-2′-deoxyuridine, *p*-nitrophenyl β-D-glucopyranoside (pNPGlu) and commercial β-galactosidase from bovine liver were obtained from Sigma–Aldrich (USA). *p*-Nitrophenyl β-D-galactopyranoside (pNPGal) and ethyl 1-thio-β-D-galactopyranoside were from Beijing Chemsynlab Pharmaceutical Science & Technology Co. (China). *o*-Nitrophenyl β-D-galactopyranoside (oNPGal) was bought from Guangzhou Genebase Bioscience Co. (China). *p*-Nitrophenyl β-D-fucopyranoside (pNPF), *p*-nitrophenyl β-D-xylopyranoside, *p*-nitrophenyl β-D-glucuronide and *p*-nitrophenyl α-D-glucopyranoside were purchased from TCI (Japan). D_2O and DMSO-d_6 were from Merck (Germany). Molecular weight marker proteins for sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were obtained from Dalian TaKaRa (China). The gel filtration calibration kit (high molecular weight) for molecular weight determination was purchased from GE Healthcare (USA). All other chemicals were from commercial sources and of the highest purity available.

2.2. β-Galactosidase activity assay

Citric acid–Na_2HPO_4 buffer (1 mL, 100 mM, pH 7.0) containing pNPGal (10 mM) was preincubated at 40 °C and 200 rpm for 10 min; then, the purified enzyme was added to initiate the reaction. After 5 min, the reaction was quenched by adding Na_2CO_3 solution (3 mL, 0.2 M) and the absorbance at 402 nm was recorded by a UV–vis spectrophotometer (Shimadzu 2550, Japan). A reaction mixture without the enzyme was used as the control. One unit of enzyme activity was defined as the amount of enzyme that releases 1.0 μmol of *p*-nitrophenol per minute under the above-mentioned conditions.

2.3. Substrate specificity

To initiate the reaction, the purified enzyme was added to citric acid–Na_2HPO_4 buffer (1 mL, 50 mM, pH 7.0) containing the substrate (2 mM) at 40 °C.

2.4. General procedure for enzymatic fucosylation of nucleosides

In a 15 mL Erlenmeyer flask with a septum cap, citric acid-Na_2HPO_4 buffer (2 mL, 50 mM, pH 7.0) containing nucleoside (0.03 mmol), pNPF (0.01 mmol) and enzyme (0.208 U) was incubated at 200 rpm and 40 °C. Aliquots were withdrawn at specified time intervals from the reaction mixture. The samples were treated at 100 °C for 5 min to denature the enzyme, and then diluted by 25 times prior to HPLC analysis. All the experiments were carried our in duplicate.

2.5. HPLC analysis

The mixture of enzymatic fucosylation was analyzed by HPLC on an XBridge C18 column (4.6 × 250 mm, 5 μm, Waters) using a Waters 1525 pump and a UV detector 2489 with a flow rate of