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High-yield enzymatic synthesis of *O*-allyl β-D-galactopyranoside

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Abstract

A high-yield synthesis of *O*-allyl β-D-galactopyranoside was carried out by the use of *Aspergillus oryzae* β-galactosidase. The reaction was carried out employing *p*-nitrophenyl β-D-galactopyranoside as the donor and a large excess of allyl alcohol as the acceptor. The molar yield was 65.6%, corresponding to an improvement of 41.3% with respect to the best results previously reported with other systems, and of 80.2% with respect to the results obtained using the same enzyme.
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1. Introduction

Allyl glycosides are useful precursors for designing new glycoconjugates and polymers, as demonstrated by different workers [1–3]. Furthermore, allyl group can be used for protecting the anomeric hydroxyl group of sugars [4]. Among the various strategies that have been proposed for the synthesis of allyl glycosides, preparations based on the use of enzymes are very simple and highly stereospecific. This paper presents a new enzymatic preparation of *O*-allyl β-D-galactopyranoside, promoted by *Aspergillus oryzae* β-galactosidase using *p*-nitrophenyl β-D-galactopyranoside as the donor and allyl alcohol as the acceptor.

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2. Experimental

2.1. Chemicals

p-Nitrophenyl β-D-galactopyranoside, sodium tetraborate (borax), sodium dodecylsulfate (SDS), *A. oryzae* β-galactosidase and allyl alcohol were from Sigma–Aldrich (St. Louis, MO, USA); Bio-Gel P2 was from Bio-Rad (Hercules, CA, USA).

2.2. Kinetis of the synthesis of *O*-allyl *β*-D-*galactopyranoside*

A total of 0.033 mmol of *p*-nitrophenyl β-D-galactopyranoside and 0.003 mol of allyl alcohol were dissolved in 1 ml of 50 mM acetate buffer, pH 4.0, and 20 ml of a *A. oryzae* β-galactosidase solution (1 mg/ml) were added. During incubation at 37 °C, at 1 h intervals, 50 ml of each sample were collected, added to 450 ml of distilled water and the mixture was

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heated in a boiling water bath for 10 min to inactivate the enzyme. Each sample was then freeze-dried, re-solubilized in 500 ml of water, and analyzed as such by capillary electrophoresis.

2.3. Synthesis and purification of O-allyl β-D-galactopyranoside

A total of 0.33 mmol of *p*-nitrophenyl β-D-galactopyranoside and 0.03 mol of allyl alcohol were dissolved in 10 ml of 50 mM acetate buffer, pH 4.0, and 200 ml of a *A. oryzae* β-galactosidase solution (10 mg/ml) were added. After incubation at 37 °C for 3 h, the mixture was heated in a boiling water bath for 10 min to inactivate the enzyme. The mixture was purified on a column (2.0 cm × 100 cm) of Bio-Gel P2 equilibrated in water.

2.4. Capillary electrophoresis

The system was an Applied Biosystems HPCE Model 270A-HT with Turbochrom Navigator (4.0) software. The fused silica column (72 cm (50 cmtodetector) × 50 μm i.d. × 375 μm o.d.) was from Supelco (St. Louis, MO, USA). All runs were done at 30 °C. Samples were loaded under vacuum at a pressure of 16.9 kPa (1.5 s). Before sample injection, a 4 min conditioning of the capillary with the buffer followed a 2 min washing with 0.1N NaOH (vacuum pressure 67.6 kPa). MEKC-UV was performed using 100 mM borax + 100 mM SDS (15 kV; 195 nm).

2.5. Mass spectrometry

Mass spectra were recorded on an API-I PE SCIEX quadrupole mass spectrometer equipped with an articulated ion spray connected to a syringe pump for sample injection. The solvent was 50% aqueous methanol containing formic acid (0.1%). The injection flow rate was equal to 0.1 ml/h; the ionspray voltage was 5600 V.

2.6. Nuclear magnetic resonance

^{13}C NMR spectra were recorded on a JEOL EX400 (100.6 MHz) spectrometer. All NMR experiments were carried out in D_2O.

3. Results and discussion

The time course of the enzymatic synthesis of *O*-allyl β-D-galactopyranoside promoted by *A. oryzae* β-galactosidase was followed using micellar capillary electrophoresis (MEKC) with UV detection. Portions of the synthesis mixtures were collected at 1 h intervals and injected as such in the capillary electrophoresis (CE) system, after inactivation of the enzyme. In Fig. 1, the MEKC analysis [6] of the synthesis mixture (after 3 h of reaction) is reported. Tetraborate (100 mM) formed charged complexes with the saccharidic compounds present in the synthesis mixture; such complexes also behaved as a suitable chromophore [7,8]. Sodium dodecylsulfate (50 mM) improved the separation between *O*-allyl β-galactopyranoside and *p*-nitrophenyl β-D-galactopyranoside. The molar yield of synthesis *O*-allyl β-D-galactopyranoside as a function of the reaction time, as obtained by MEKC analysis, is shown in Fig. 2. The curve reached a plateau after about 3 h. The amount of product isolated by size-exclusion chromatography was calculated by using a calibration plot (peak area/migration time versus molar concentration). The linear range extended to 20 mM, with r^2 equal to 0.9997 (slope: 1.33; intercept: 0.000150). The electrospray mass spectrum of the purified allyl-galactoside evidenced the presence of peaks at m/z = 221.2, 238.2, 243.2 and 259.2, corresponding to $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, $[M+K]^+$, respectively (data not shown). Table 1 reports the ^{13}C NMR data, which are in agreement with those reported in literature [3,9]; the chemical structure of the product was then definitively assessed. The molar yield of the synthesis reaction, evaluated by MEKC, was equal to 70%, while the molar yield calculated on the basis of the isolated product was

Table 1
^{13}C NMR data for *O*-allyl β-D-galactopyranoside

C-1	102.0
C-2	70.9
C-3	73.0
C-4	68.8
C-5	75.3
C-6	61.1
O$\underline{C}H_2CH{=}CH_2$	70.8
O$CH_2\underline{C}H{=}CH_2$	133.6
O$CH_2CH{=}\underline{C}H_2$	118.8

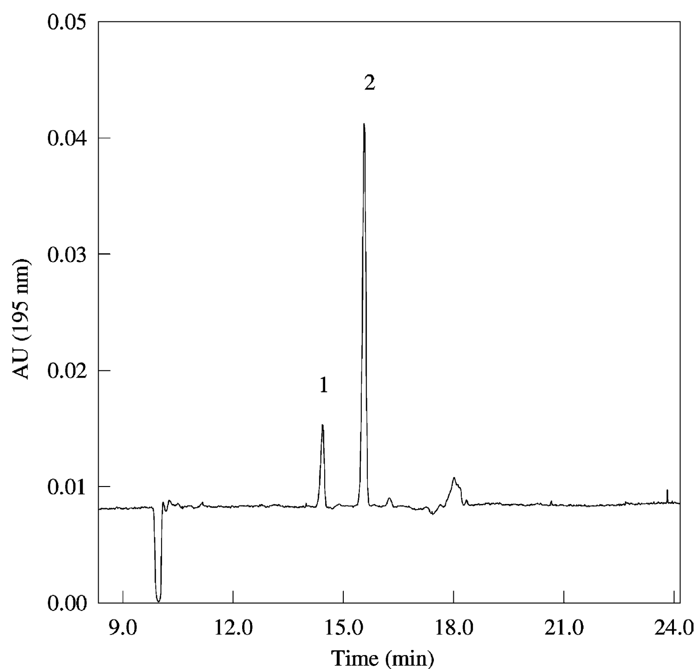

Fig. 1. MEKC-UV analysis of untreated *O*-allyl β-D-galactopyranoside synthesis mixture. Buffer: 100 mM borax + 50 mM SDS. Peak attributions: (1) *O*-allyl β-galactopyranoside; (2) *p*-nitrophenyl β-galactopyranoside.

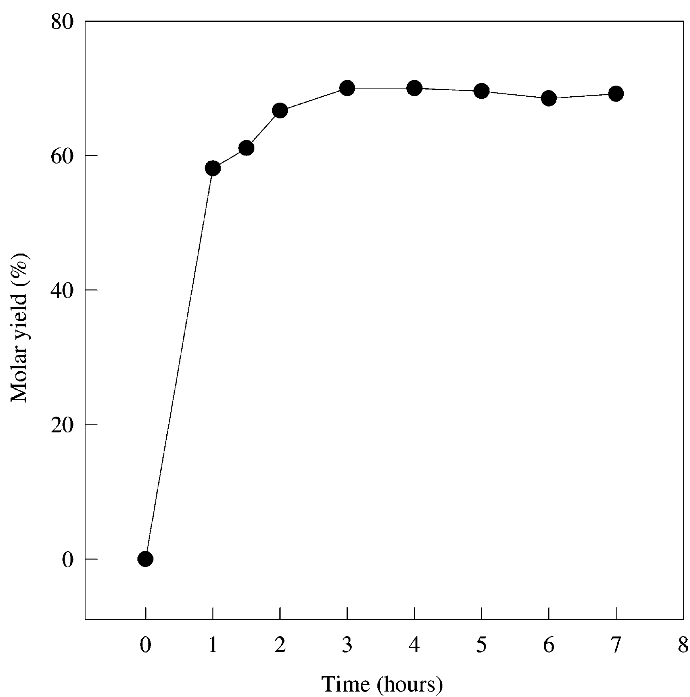

Fig. 2. Time-course of the production of *O*-allyl β-D-galactopyranoside during 7 h of incubation at 37 °C.

equal to 65.6%. The discrepancy between molar yield calculated is reasonably due to the loss of product during purification. The yield of the reaction was significantly improved with respect to the previously reported syntheses, in which lactose was used as the donor, allyl alcohol as the acceptor. The used enzymes were *A. oryzae* β-D-galactosidase (yield: 13% [3]), *E. coli* β-D-galactosidase (yield: 30% [5]), *Streptococcus thermophilus* β-D-galactosidase (yield: 38.5% [4]). Another approach exploited the action of almond β-glucosidase, using galactose as the donor and allyl alcohol as the acceptor and as the solvent (containing 10% of water); the yield in this case was 15% [9]. Lactose and galactose then clearly seems to give yield values lower than those of *p*-nitrophenyl β-D-galactopyranoside [3–5,9]. The less expensive allyl alcohol was used in large excess with respect to the more expensive *p*-nitrophenyl β-D-galactopyranoside (molar ratio 91:1); the increase in yield, however, makes the reaction presented here suitable for scale-up. The present results demonstrate that the structure of the aglycon portion of donors plays an important role to achieve a high yield for the transglycosylation reactions, as pointed out also in a previous publication [10]. The preliminary nature of this work, however, does not allow to safely highlight the specific contributions to the observed effect.

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