One-Pot Synthesis of 1-Octyl β -D-Glucoside from Cellulose and 1-Octanol in Combination of Cellulase and β -Glucosidase

Shuichi MATSUMURA, Hiroshi TSURUTA, and Kazunobu TOSHIMA

Department of Applied Chemistry, Faculty of Science and Technology, Keio University
(3-14-1, Hiyoshi, Kohoku-ku, Yokohama-shi 223-8522)

Abstract : 1-Octyl β -D-glucoside was prepared by one-pot reaction of cellulose and 1-octanol using the combination of cellulase and β -glucosidase. In combination with 0.1% cellulase from *Aspergillus niger* and 0.5% β -glucosidase from Almonds, 1-octyl β -D-glucoside was produced by as much as 245 mg/g cellulose during the incubation of 1-octanol saturated with 100 mM acetate buffer (pH 5.0) and α -cellulose at 37°C for 5 d.

Key words : octyl glucoside, nonionic surfactant, cellulase and β -glucosidase, cellulose, one-pot enzymatic synthesis

1 Introduction

Carbohydrate is one of the alternative feedstocks for the preparation of surfactants because it is an inexpensive, renewable and biodegradable raw material. Among the carbohydrates, cellulose is the most abundant, renewable natural polymer on the earth.

Alkyl glycosides, such as octyl glucoside, are of particular interest because, in addition to their potential biological and pharmaceutical applications^{1)–12)}, they are made from naturally occurring renewable resources of fatty alcohols and sugars. Furthermore, from an ecological point of view as well as an energy consideration, alkyl glycosides will be some of the most promising candidates of new surfactants in the next generation. Recently, alkyl polyglycosides have been commercially produced from monomeric D-glucose and a fatty alcohol in the presence of a catalyst, and they have been proven to show excellent properties of both surfactants and biodegradability. However, they are a mixture of several homologous and isomeric compounds, which are not suited for biological and pharmaceutical applications. Furthermore, the conventional preparation of alkyl glycosides was carried out using monomeric sugar units which are obtained from polysaccharides, such as starch, by hydrolysis. The direct reaction of polysaccharides

and a fatty alcohol may be advantageous for industrial production because the hydrolysis of polysaccharides and the purification process for monomeric sugar will be omitted. Under these circumstances, the enzymatic synthesis of alkyl glycosides will be the more attractive method.

The enzymatic synthesis of alkyl glycosides, such as alkyl glucoside^{13)–18)}, alkyl galactoside^{19)–21)} and alkyl xyloside²²⁾ have been reported. Recently, an alkyl oligoxyloside could be prepared by the one-pot reaction of xylan and a fatty alcohol using microbes. We previously reported the one-pot enzymatic synthesis of alkyl xylobioside and xyloside using a xylan-assimilating strain, *Aureobasidium pullulans* KK415 (ATCC 201145), as the enzyme source^{23,24)}. Also, heptyl xyloside was prepared by the one-pot reaction of xylan and heptanol using the culture broth of *Penicillium* sp. by Shinoyama *et al.*²⁵⁾. However, the one-pot synthesis of alkyl glycosides using cellulose has not been reported.

This paper describes the one-pot synthesis 1-octyl β -D-glucoside from α -cellulose and 1-octanol using cellulase and β -glucosidase.

2 Experimental

2.1 Materials and measurements

α -Cellulose was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). 1-Octyl β -D-glucoside as the authentic sample was prepared according to a previous paper⁹⁾. Cellobiose was purchased from Tokyo Kasei Kogyo Co.,

Corresponding author : Shuichi MATSUMURA

Ltd.(Tokyo, Japan). The other materials were of the highest available purity and used as purchased unless stated otherwise. Cellulase (EC 3.2.1.4) from *Aspergillus niger* (0.45 unit/mg solid, according to the supplier) and β -glucosidase (EC 3.2.1.21) from Almonds (5.5 unit/mg solid, according to the supplier) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). The enzymes were used without further purification. The 1-octyl β -D-glucosides were analyzed using high-performance liquid chromatography (HPLC) with a refractive index detector (RI) and a commercial HPLC column (Inertsil ODS-2, GL Sciences Inc., Tokyo, Japan, acetonitrile/water = 3/2 (v/v) as the eluent) calibrated with the chemically synthesized authentic standard. The ^1H and ^{13}C NMR spectra were recorded using a JEOL model GSX-270 Fourier Transform Spectrometer (Tokyo, Japan) operating at 270 and 67.5 MHz, respectively, with complete proton decoupling in CD_3OD at 25 °C.

2·2 Preparation of 1-octanol saturated with acetate buffer¹⁴⁾

A mixture of 100 mM acetate buffer (pH 5.0) and 1-octanol was preequilibrated with stirring overnight at 25°C. The heterogeneous mixture was then centrifuged at 3000 rpm for 5 min to obtain a transparent supernatant as the buffer saturated 1-octanol for the enzymatic reaction.

2·3 Preparation of 1-octyl β -D-glucoside

1-Octyl β -D-glucoside was prepared by the incubation of cellulose and 1-octanol in the presence of cellulase and β -glucosidase as shown in Scheme 1. A typical preparation procedure is as

follows. A mixture of α -cellulose, 1-octanol saturated with 100 mM acetate buffer (pH 5.0) and the enzymes was incubated in a glass tube with stirring using a magnetic stirring bar at 37 °C. The yield of the 1-octyl β -D-glucoside was periodically measured as follows. An aliquot (1.0 mL) of the reaction mixture was filtered through a celite pad to remove the enzyme and unreacted α -cellulose. The celite pad was then washed with ethanol. A small amount of water was added to the combined filtrate and repeatedly azeotropically evaporated in a vacuum to give the crude product. The crude product was directly analyzed by HPLC using the authentic standard. For the structure analysis, the crude product was purified by silica gel column chromatography [$(\text{SiO}_2, \text{CHCl}_3/\text{CH}_3\text{OH} = 3/1 \text{ (v/v)})$] to give 1-octyl β -D-glucoside [$R_f = 0.6, \text{CHCl}_3/\text{CH}_3\text{OH} = 3/1 \text{ (v/v)}$]. The structures of these compounds were confirmed by HPLC, elemental analysis, and ^1H NMR and ^{13}C NMR spectroscopies. 1-Octyl β -D-glucoside: Elemental analysis, Found : C, 57.65 ; H, 9.78%. Calculated for $\text{C}_{14}\text{H}_{28}\text{O}_6$: C, 57.51 ; H, 9.65%. ^1H NMR (270 MHz : CD_3OD) : δ 0.88 (3H, t, $J=4.0$ Hz), 1.25 (10H, m), 1.52 (2H, m), 3.00-3.87 (8H, m), 4.16 (1H, m). ^{13}C NMR (67.5 MHz : CD_3OD) : δ 14.4, 23.7, 27.1, 30.4, 30.6, 30.8, 33.0, 62.7, 70.9, 71.6, 75.1, 77.8, 78.1, 104.3.

3 Results and Discussion

It was found that 1-octyl β -D-glucoside was produced using a combination of cellulase and β -glucosidase by the one-pot synthesis of cellulose

Scheme 1

Table 1 Enzymatic Synthesis of 1-Octyl β -D-glucoside.

Substrate	Enzyme	Enzyme conc. (%)	Time(h)	Yield (mg/g substrate)
D-glucose	β -glucosidase	0.05	16	120
cellobiose	cellulase	0.5	16	18
cellobiose	β -glucosidase	0.05	16	114
α -cellulose	cellulase	0.5	16	1
α -cellulose	{ cellulase β -glucosidase	{ 0.5 0.1	16	125

0.33 % D-Glucose, 0.33 % cellobiose or 0.33 % cellulose was reacted with 1-octanol saturated with 100 mM acetate buffer (pH 5.0, 15 mL) using cellulase, β -glucosidase or a combination of cellulase and β -glucosidase at 37°C for 16 h with stirring.

and 1-octanol in a maximum yield of 245 mg/g cellulose. **Table 1** shows some yields of the 1-octyl glucoside obtained by the reaction of α -cellulose, cellobiose or D-glucose with cellulase, β -glucosidase or a combination of cellulase and β -glucosidase. It was confirmed that octyl glucoside was produced by the reaction of D-glucose or cellobiose with β -glucosidase, or α -cellulose with a combination of cellulase and β -glucosidase. Though a small amount of 1-octyl β -D-glucoside was produced by cellulase without β -glucosidase, this may be ascribed to the contaminated β -glucosidase in the cellulase. As measured using p-nitrophenyl β -D-glucoside, the β -glucosidase activity of the cellulase was 0.57 unit/mg solid.

Fig. 1 shows the time course of the yield of 1-octyl β -D-glucoside from the reaction of 0.33% α -cellulose and 1-octanol saturated with 100 mM acetate buffer (pH 5, 15 mL) using a combination

of 0.1–0.5% cellulase and 0.1% β -glucosidase. The yield of octyl glucoside gradually increased and reached a maximum value after 5 d depending on the concentration of the cellulase. The yield of the octyl glucoside remained almost constant after this 5-d incubation.

Fig. 2 shows the effects of cellulase concentration on the yield of 1-octyl glucoside. It was found that the maximum yield was obtained when 0.5% cellulase and 0.1% β -glucosidase (weight ratio of cellulase and β -glucosidase being 5/1) was used. Both the higher and lower concentrations of cellulase decreased the yield of octyl glucoside when the concentration of β -glucosidase remained constant at 0.1%.

Fig. 3 shows the effects of enzyme concentration on the yield of the octyl glucoside. It was observed that the octyl glucoside yield was slightly increased with increasing enzyme concentration up to about 0.6% when the weight ratio of cellulase and β -glucosidase was 5/1. However, the differ-

Fig.1 Time Course of 1-Octyl β -D-glucoside by the Reaction of 0.33 % Cellulose and 1-Octanol Saturated with 100 mM Acetate Buffer (pH 5.0, 15 mL) Using 0.1–0.5 % Cellulase and 0.1 % β -Glucosidase at 37°C.

Cellulase : ○ : 0.5 %, ● : 0.4 %, △ : 0.3 %, ▲ : 0.1 %

Fig.2 Effects of Cellulase Concentration on the Yield of 1-Octyl β -D-Glucoside by the Reaction of 0.33 % Cellulose and 1-Octanol Saturated with 100 mM Acetate Buffer (pH 5.0, 15 mL) Using 0.1–0.6 % Cellulase and 0.1 % β -Glucosidase at 37°C for 5d.

Fig. 3 Effects of Enzyme Concentration on the Yield of 1-Octyl β -D-Glucoside by the Reaction of 0.33 % Cellulose and 1-Octanol Saturated with 100 mM Acetate Buffer (pH 5.0, 15 mL) Using a Mixed Enzyme of Cellulase/ β -glucosidase Having a Weight Ratio of 5/1 at 37°C for 5d.

ence in the enzyme concentration between 0.1 and 0.75% did not significantly affect the glucoside yield.

The concentration of cellulose was the decisive factor governing the octyl glucoside yield. **Fig. 4** shows the effects of cellulose concentration on the octyl glucoside yield. The octyl glucoside yield quickly decreased when the cellulose concentration was higher than about 0.3% for the reaction of cellulose and 1-octanol saturated with 100 mM acetate buffer (pH 5.0, 15 mL) using 0.5% cellulase and 0.1% β -glucosidase at 37 °C for 5 d. The decrease in the octyl glucoside yield was

Fig. 4 Effects of Cellulose Concentration on the Yield of 1-Octyl β -D-Glucoside by the Reaction of Cellulose and 1-Octanol Saturated with 100 mM Acetate Buffer (pH 5.0, 15 mL) Using 0.5 % Cellulase and 0.1 % β -Glucosidase at 37°C for 5d.

ascribed to the depletion of water for effective hydrolysis of the cellulose by cellulase when an excess amount of cellulose was used.

4 Conclusions

It was found that 1-octyl β -D-glucoside was produced from cellulose and 1-octanol by the action of the combined enzyme system of cellulase and β -glucosidase during a one-pot synthesis. In combination with 0.1% cellulase from *Aspergillus niger* and 0.5% β -glucosidase from Almonds, 1-octyl β -D-glucoside was produced by as much as 245 mg/g cellulose during the incubation of α -cellulose and 1-octanol saturated with acetate buffer (pH 5.0) at 37 °C for 5 d.

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日本油化学会誌本号掲載 論文要旨

[総説] コレステロールオキシダーゼの特性とその応用

北 本 大

通産省工業技術院 物質工学工業技術研究所 (〒305-8565 つくば市東 1-1)

コレステロールオキシダーゼ (CO) は、コレステロール代謝の鍵酵素であり、ステロイドの 3 位 β 水酸基の酸化、5 位二重結合の 4 位への異性化を同時に行う 2 機能性酵素である。CO は *Brevibacterium*, *Nocardia*, *Psuedomonas*, *Rhodococcus* や *Streptomyces* 属等の多くの土壤微生物によって生産され、種々の分野で利用されている産業用酵素である。今日では、血清中のコレステロールの定量試薬として臨床検査キットなどに広く使われている。

CO は広い基質特異性を有しており、コレステロール以外の多種のステロイド 3 位 β 水酸基に作用する。CO は有機溶媒中でも安定であり、また最近ステロイド系以外の化合物の位置選択的、立体選択的酸化にも利用され、リパーゼのような日常的な有機合成試薬としての利用が期待されている。*Brevibacterium* 属由来の CO の 3 次元構造は既に明らかになっているが、その詳細な反応機構に関しては未だ十分な情報は得られていない。

CO はワタノハナゾウムシなどの害虫に対し強い殺虫活性を持つことが最近明らかになり、害虫抵抗性作物の育種のため CO 遺伝子の植物細胞での発現が検討されている。また、チーズやヨーグルトなどの発酵食品中のコレステロール含量を低減させる目的で、CO 遺伝子の乳酸菌での発現も研究されている。

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[報文] セルラーゼと β -グルコシダーゼの組み合わせによる
セルロースと 1-オクタノールからの 1-オクチル
 β -D-グルコシドのワンポット合成

松村 秀一・鶴田 浩史・戸嶋 一敦

慶應義塾大学理工学部 (〒223-8522 横浜市港北区日吉 3-14-1)

セルラーゼと β -グルコシダーゼを組み合わせたワンポット合成によって、セルロースとオクタノールからオクチル β -D-グルコシドが得られた。100 mM 酢酸緩衝液 (pH 5.0) で飽和した 1-オクタノールと α -セルロースを *Aspergillus niger* 起源の 0.1% セルラーゼ及びアーモンド起源の 0.5% β -グルコシダーゼ存在下、37℃で 5 日間反応を行うことにより、1-オクチル β -D-グルコシドが収率 245 mg/g cellulose で得られた。

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