Enzymatic synthesis of methyl β-D-glucoside directly from cellulose pretreated with biocompatible amino acid ionic liquid/cosolvent

Juan Tao, Takao Kishimoto*, Masahiro Hamada and Noriyuki Nakajima

Abstract: A new approach for the enzymatic synthesis of methyl β-D-glucoside was proposed, based on commercially available cellulase and cellulose pretreated with phosphonium-based amino acid ionic liquid/cosolvent. The pretreatments were quite effective and methyl β-D-glucoside was successfully synthesized with 40% yield from cellulose (Avicel) pretreated with tetrabutylphosphonium glycine/dimethyl sulfoxide (DMSO), whereas the yield was only 1.4% from untreated cellulose. Preparative-scale synthesis from 1 g cellulose with a reduced amount of cellulase was also conducted, achieving a 33% isolated yield. Results of additional studies with cellobiose and glucose as substrates have been interpreted as cellulose being first enzymatically hydrolyzed to cellobiose, which then reacted with methanol to produce methyl β-D-glucoside by transglycosylation.

Keywords: amino acid ionic liquids, cellulose, enzymatic synthesis, methyl β-D-glucoside

Introduction

Cellulose is the most abundant renewable resource on the earth, and its utilization as a high-value-added product is of great importance. However, the poor solubility of cellulose in common solvents is the main obstacle to its further application. Ionic liquids (ILs) with negligible vapor pressure, strong solubilization efficiency, adjustable structure, and recyclability have been widely applied to lignocelluloses (Swatloski et al. 2002; Zhang et al. 2005; Fukaya et al. 2006; Qu et al. 2011, 2012, 2013a,b; Tao et al. 2016a). After pretreatments with ILs, the highly crystalline structure of cellulose is altered to a disordered state with a more accessible surface area (Auxenfans et al. 2012; Xiao et al. 2012), which provides the possibility of transforming cellulose into advanced derivatives.

Alkyl glucosides are biodegradable nonionic surfactants that have been applied in many fields, including detergents, cosmetics, food, and pharmaceuticals (Matsumura et al. 1990). Methyl β-D-glucoside, one of the short-chain alkyl glucosides, has been used to synthesize long-chain alkyl glucosides by transacetalization (Papanikolaou 2001; Rather et al. 2012). Methyl β-D-glucoside is a good model compound to elucidate the reaction mechanisms of cellulose (Kruså et al. 2005; Carlsson et al. 2006; Fan et al. 2015).

Traditional glycosylation methods are chemical processes that require complex protection and deprotection of hydroxy groups to obtain the desired products (Schmidt 1986). In addition, these processes often require strong acid or acid resin (Brochette et al. 1997; Ignatyev et al. 2010; Villandier and Corma 2010; Dora et al. 2012). The enzymatic synthesis of alkyl glucosides has received a great deal of attention for producing desired products in one step (Vic and Thomas 1992; Yi et al. 1998; Matsumura et al. 1999; Ducret et al. 2002; Svasti et al. 2003; Vijayakumar et al. 2007; Rather et al. 2010). However, despite numerous studies of enzymatic methods, cellulose is rarely used as a starting material. Glucose, cellobiose, p-nitrophenyl β-D-glucopyranoside (pNPG), and other soluble carbohydrates are more common in synthetic processes, probably because of their greater accessibility to enzymes.

Consequently, the purpose of this study was to use cellulose as a starting material to enzymatically synthesize methyl β-D-glucoside. Eco-friendly phosphonium-based amino acid ionic liquid (AAIL)/cosolvent systems served as cellulose pretreatment solvents, which recently

*Corresponding author: Takao Kishimoto, Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, Imizu 939-0398, Japan, e-mail: takao@pu-toyama.ac.jp

Juan Tao, Masahiro Hamada and Noriyuki Nakajima: Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, Imizu 939-0398, Japan
demonstrated higher compatibility with cellulase than imidazolium- and halogen-based ILs (Tao et al. 2016b). The experimental design was aimed at the utilization of commercial cellulase without any modification. Moreover, the effect of IL pretreatments and methanol concentrations should be evaluated on the formation of methyl β-D-glucoside. The mechanism of methyl β-D-glucoside formation from cellulose will be discussed and a preparative-scale synthesis should be performed aiming at the reduced amount of cellulase.

### Materials and methods

Microcrystalline cellulose, Avicel PH-101 (Sigma-Aldrich Co., St. Louis, MO, USA), was dried under vacuum over phosphorus pentoxide (P2O5) at room temperature (r.t.) for 24 h. All other chemicals (Wako Pure Chemical Industries, Osaka, Japan) were reagent grade or higher and used without purification. Cellulase “Onozuka” RS was purchased from Yakult Pharmaceutical Industry Co., Ltd., Tokyo, Japan. The optimum pH was between 4.0 and 5.0, and the optimum temperature was 50°C–60°C (Tao et al. 2016b). Tetraethylphosphonium glycine ([TBP][Gly]) was prepared as described by Tao et al. (2016b).

### Enzymatic synthesis of methyl β-D-glucoside from cellulose:

Cellulose (18 mg) was mixed with IL/cosolvent (1:1, w/w, 180 mg) in a screw-capped vial, and stirred magnetically at 30°C for 30 min. Distilled water (1.4 ml) was added with vigorous stirring for 30 min. The suspension was centrifuged at 4000 rpm for 20 min and then the regenerated cellulose was washed with water (1.4 ml) three times to remove the residual IL and cosolvent. Acetate buffer (0.1 M, pH 4.5) and 13.5%–27% MeOH (v/v) were added to a total volume of 2 ml. The enzymatic synthesis was performed with agitation (200 rpm) at 50°C by adding 2.1 mg of cellulase (with a protein content of 0.5 mg). At given time intervals, 50 μl of the reaction solution was withdrawn, 50 μl of the reaction solution was withdrawn, and heated at 105°C for 5 min to inactivate cellulase. All experiments were conducted in triplicate.

### Results and discussion

#### Enzymatic synthesis of methyl β-D-glucoside

It was already demonstrated that TBP-based AAIL/cosolvent is a new effective pretreatment solvent for enhanced enzymatic hydrolysis of cellulose because of its higher compatibility with cellulase (Tao et al. 2016b). Hence, phosphonium-based AAIL/cosolvent systems were further applied to the synthesis of methyl β-D-glucoside from regenerated cellulose and MeOH. Cellulose was pretreated with combinations of [TBP][Gly] with cosolvent, DMSO, N-methylimidazole (NMI), or N-methyl-2-pyrroldione (NMP), as illustrated in Figure 1. For comparison, 1-allyl-3-methylimidazolium chloride ([Amim][Cl])/DMSO served as a conventional IL. The MeOH content was 22.5% (v/v) in acetate buffer (pH 4.5), which corresponds to a substrate (glucose residue)-to-MeOH ratio of 1:125.

From the original cellulose (without pretreatment), methyl β-D-glucoside was not formed effectively (Figure 1a), along with quite low yields of glucose (Figure 1b) and cellobiose (Figure 1c). A total enzymatic conversion of only 6% was observed in the presence of...
rates reached 85%–86%, which were much higher than those of untreated cellulose because hydrogen bonding of the original cellulose was altered by the IL/cosolvent pretreatments and the disordered cellulose structure was more accessible to cellulase (Cui et al. 2014; Mai et al. 2014). In addition, pretreatments with [TBP][Gly]/cosolvent were more efficient, and the yields of methyl \( \beta \)-d-glucoside were approx. 10% higher than those by [Amim]Cl/DMSO, which can be attributed to the higher compatibility of residual [TBP][Gly] with cellulase than that of residual [Amim]Cl in regenerated celluloses (Tao et al. 2016b). It is also notable that among the cosolvents observed, DMSO was the most effective one for the synthesis of methyl \( \beta \)-d-glucoside.

**Effect of MeOH contents on the synthesis**

Methanol was the main reactant in the enzymatic synthesis in focus. However, as pointed out, MeOH has clear harmful effects on cellulase activity. Hence, it is essential to optimize the MeOH content for the enzymatic synthesis of methyl \( \beta \)-d-glucoside. Cellulose pretreated by [TBP][Gly]/DMSO was washed with water and subjected to enzymatic treatments in acetate buffer with 13.5%–27% (v/v) MeOH (Figure 2). The yield of methyl \( \beta \)-d-glucoside clearly increased with increasing MeOH content from 13.5% to 22.5%, and a maximum methyl \( \beta \)-d-glucoside yield of 40% was observed upon incubation for 24 h (Figure 2a). Higher MeOH contents led to more interaction with cellulose, as reflected by the higher methyl \( \beta \)-d-glucoside yield. However, a significant decrease in the yield of methyl \( \beta \)-d-glucoside was observed for 27% (v/v) MeOH. In contrast, the glucose yields showed a reverse tendency and were highest for 13.5% MeOH. The total conversion rates of regenerated cellulose were relatively constant between 85% and 95% for MeOH contents of 13.5%–22.5%. For MeOH contents of 13.5% and 18%, the formation of methyl \( \beta \)-d-glucoside appeared to be completed in 12 h, and a decrease in the yield of methyl \( \beta \)-d-glucoside was observed, as shown in Figure 2a. This indicates that with prolonged incubation, the hydrolysis of methyl \( \beta \)-d-glucoside to glucose became pronounced, particularly at low MeOH contents.

**Exploration of the formation of methyl \( \beta \)-d-glucoside**

It was particularly significant to determine how methyl \( \beta \)-d-glucoside is formed by cellulase from insoluble
cellulose, because this is the first study with this regard. In light of the synthesis of alkyl β-D-glucoside with almond β-glucosidase and soluble glucose (Papanikolaou 2001; Ducret et al. 2002), we estimated that glucose formed from cellulose might act as a glycosyl donor. Thus, regenerated cellulose was first enzymatically hydrolyzed by cellulase to glucose for 4 h, and then MeOH was added and the incubation was continued for an additional 44 h, as shown in Figure 3. Surprisingly, a high glucose level (83% at 4 h) was maintained, with only a slight decrease during incubation, while only a limited amount of methyl β-D-glucoside was formed. Thus, the glucose formed from cellulose was not a main precursor for the reaction in focus.

Next, cellobiose and glucose, which are the main products from the saccharification of cellulose, were selected as substrates for the synthesis under the same incubation conditions. As shown in Figure 4, the formation of methyl β-D-glucoside from cellobiose followed a time course similar to that from regenerated cellulose, associated with a higher initial formation rate and higher yields. In contrast, the rate of conversion of glucose into methyl β-D-glucoside was quite low, particularly during the first 4 h. These results are interpreted as cellulose being first enzymatically hydrolyzed to cellobiose and then the formed cellobiose being converted into methyl β-D-glucoside by transglycosylation (Figure 5). The transglycosylation of cellobiose would produce both methyl β-D-glucoside and...
glucose at a 1:1 ratio. At the same time, cellobiose was also simply hydrolyzed to glucose in this highly aqueous solution. This explains why, despite a high total conversion of regenerated cellulose (>85%), methyl \(\beta\)-d-glucoside was obtained at a relatively lower yield (<44%). Further research is necessary to achieve a higher conversion rate of cellulose to methyl \(\beta\)-d-glucoside.

**Preparative-scale synthesis of methyl \(\beta\)-d-glucoside from cellulose**

Preparative-scale synthesis of methyl \(\beta\)-d-glucoside was performed under the same conditions as in the case of analytical experiments, but the amount of cellulase was reduced. Compared with the small-scale experiments, the initial production rates for both methyl \(\beta\)-d-glucoside and glucose clearly decreased because of the small amount of cellulase, as shown in Figure 6. However, the yields of methyl \(\beta\)-d-glucoside and glucose after 48 h of incubation were 36% and 46%, respectively, which were comparable to the yields for the small-scale synthesis, namely, 40% and 46%, respectively. Methyl \(\beta\)-d-glucoside could be isolated by column chromatography at a 33% isolated yield.

**Conclusions**

Pretreatments with AAIL/cosolvent were essential for the enzymatic synthesis of methyl \(\beta\)-d-glucoside from cellulose, and the initial formation rates and yields increased dramatically. The methanol content was one of the key factors for the effective synthesis because methanol is not only an important reactant but also has an inhibitory effect on cellulase activity. Exploration of the formation route provides valuable insights into the highly efficient synthesis of methyl \(\beta\)-d-glucoside from cellulose.

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**References**


