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Microwave-assisted synthesis of butyl galactopyranoside catalyzed by β-galactosidase from *Thermotoga naphthophila* RKU-10

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ABSTRACT

Microwave-assisted enzymatic synthesis of butyl galactopyranoside was performed in this study. Microwave irradiation shortens reaction times, increases the productivity of the alkyl galactopyranoside reaction, and decreases the amount of enzyme required for the reaction. The optimal conditions for the enzymatic synthesis of alkyl galactopyranoside from lactose and *n*-butanol by β-galactosidase from *Thermotoga naphthophila* RKU-10 (TN1577) under a microwave heating system were determined. The influences of the molar ratio between lactose and *n*-butanol (1:200–1:40) and temperature (65–85 °C) on the transgalactosylation yield were investigated. The output power of the microwave ranged from 80 W to 800 W. Under optimal conditions (i.e., a lactose and *n*-butanol molar ratio of 1:40 and a reaction temperature of 75 °C), a yield of 17.07 mg mL^{-1} was obtained after a 3.5 h reaction incubation period. Compared with conventional heating, the microwave-assisted method improved the yield 11-fold. Microwave irradiation significantly increased the k_{cat} of β-galactosidase, but had little effect on K_m compared with conventional heating. Based on these findings, the microwave-assisted enzymatic synthesis method enables the rapid and more economical production of butyl galactopyranoside with lower time and energy costs.

1. Introduction

Microwaves are part of the electromagnetic spectrum featuring radiation with frequencies ranging from 300 MHz to 300 GHz, which correspond to wavelengths ranging from 1 m to 1 mm [1]. Studies on the interactions of microwave radiation with biological systems are an ongoing topic of both interest and controversy. Numerous studies are available concerning the effects of microwave radiation on non-thermal prokaryotic proteins [2]. Microwave radiation can cause a decline or complete loss of enzymatic activity. Nevertheless, in most cases, microwave irradiation does not significantly reduce enzymatic activity but instead improves reaction rates at low temperatures [3–5].

The depletion of nonrenewable resources and the awareness of the need for environmental protection have simultaneously increased. Thus, to address the increased demands for surfactants, new green surfactants have been developed [6,7]. Alkyl glycosides, a type of all-natural, non-toxic, non-ionic surfactant, have better features than conventional surfactants. They are low-foaming, non-irritating to the skin and eyes, and readily biodegradable, and they have a highly compatibility, a low surface tension, a wide pH adaptability, and many other unique features [8,9]. Alkyl glycosides are mainly used as cleaning chemicals and cosmetic components, and they can also be utilized for many other applications, such as industrial emulsifiers, wetting agents, thickeners, medicines, and pesticide synergists [9–12].

The major methods of alkyl glycoside synthesis include chemical and enzymatic methods, of which the enzymatic method is more important [13,14]. Numerous reports on the biosynthesis of alkyl glycoside have been published. The critical limitations of the current systems are long reaction times and poor yields [15,16]. Microwave irradiation, a green and clean alternative energy source, has recently been used to enhance the reaction rates of enzymatic synthesis [17–19]. Under microwave irradiation, the direct energy transfer between the electromagnetic field and the domains of a polar protein can induce modifications in enzyme flexibility, which consequently changes its enzymatic properties [4,20].

β-Galactosidase, which catalyzes the conversion of lactose into glucose and galactose, is an essential enzyme in the dairy industry [21]. This enzyme is broadly used to prepare lactose-hydrolysed products for lactose-intolerant or lactase-deficient

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people. In several studies, β-galactosidase was used to glycosylate small molecular compounds [22,23] and saccharides [24,25], which play crucial roles in the production industry.

In this study, an optimized strategy using thermostable β-galactosidase for microwave irradiation-catalyzed transgalactosylation to shorten reaction times was explored. The transgalactosylation of lactose with *n*-butanol by β-galactosidase from *Thermotoga naphthophila* RKU-10 (TN1577) was selected as the model reaction. The recombinant thermostable β-galactosidase was expressed in *Escherichia coli*. The purification and characterization of this enzyme has been previously described [26]. The effects of different parameters, including the reaction temperature, molar ratio of reactants, catalyst amount, microwave power, and reaction time, were studied. This study is the first to report the enzymatic synthesis of alkyl galactopyranosides by β-galactosidase under microwave irradiation at a high temperature.

2. Materials and methods

2.1. Materials

D-(+)-Lactose monohydrate was purchased from TCI (Tokyo, Japan). Silica gel plates were provided by the Qingdao Haiyang Chemical Co., Ltd. (Qingdao, China). The glucose oxidase kit used in this work was purchased from Changchun HuiLi Biotech Co., Ltd. (Changchun, China). All other chemicals used were reagent grade and purchased from Beijing DingGuo Changsheng Biotechnology Co., Ltd. (Beijing, China).

2.2. General experimental setup

2.2.1. Preparation of β-galactosidase and β-glucosidase

The same protocol was applied to produce β-galactosidase and β-glucosidase from *T. naphthophila* RKU-10. *E. coli* BL21(DE3) cells were pre-incubated in Luria–Bertani medium (LB medium, with 1 mg/mL ampicillin) with 180 rpm agitation at 37 °C. After the OD_{600} of the culture liquid reached 1.0, IPTG was added to induce enzyme expression, and the cells were grown at 25 °C for 12 h with 150 rpm agitation. The induced cells were harvested by centrifugation and washed once with 50 mM sodium phosphate buffer (pH 7.0). A 5-g dispersion of the cells in 50 mL of 50 mM sodium phosphate buffer (pH 7.0) was disrupted by sonication. The cellular debris was removed by centrifugation (15,000 × *g* for 15 min at 4 °C) to obtain a crude lysate. The crude extract was incubated in a water bath for 10 min at 80 °C to denature the *E. coli* proteins. The extract was then centrifuged to separate the crude enzyme from the heat-denatured cellular components and proteins. Finally, the extract was lyophilized for subsequent experiments.

2.2.2. β-Galactosidase activity assay

β-Galactosidase activity was measured using lactose as a substrate. The enzyme (1 mg/mL) was added to a reaction mixture containing 5% lactose and 50 mM sodium phosphate buffer (pH 7.0), with a final volume of 1 mL. The mixture was incubated at 70 °C for 30 min, which is the standard assay method. The amount of glucose produced during the incubation was assessed using a glucose oxidase kit and by observing changes in absorbance at 510 nm. One unit of enzymatic activity was defined as the amount of enzyme required to liberate 1 μmol of glucose per minute under the experimental conditions [26].

2.2.3. Conventional heating synthesis assay

The conventional heating synthesis of butyl galactopyranoside was carried out in an aqueous (sodium phosphate buffer, 50 mM, pH 7.0) organic solvent mixture (87:13, v/v) with lactose as the sugar donor and *n*-butanol as the sugar acceptor. The optimal pH is 7.0 for the conventional heating reaction (Fig. S1). The lactose and *n*-butanol molar ratio was 1:40, and 10 U of the enzyme was added to the mixture to initiate the reaction. The reaction was performed at 75 °C in a total volume of 2 mL. The water content was set to 15% in all subsequent reactions (Fig. S2).

2.2.4. Microwave-assisted enzymatic synthesis assay

The microwave experiments were carried out in a commercial multimode microwave reactor (MCR-3, Shanghai JieSi Microwave Chemistry Corporation, China). The machine has a continuous-focus microwave power delivery system with operator-selectable power outputs ranging from 0 W to 800 W. The contents of the vessel were stirred with a rotating magnetic plate located below the floor of the microwave cavity and a Teflon-coated magnetic stirring bar in the vessel. The reaction system was identical to that used for conventional heating.

2.2.5. Thermal stability of β-galactosidase under microwave irradiation

In the thermal stability tests, samples from the β-galactosidase preparation were incubated at 70, 75, or 80 °C under microwave irradiation for 1, 2 or 3 h. Samples were taken every 15 min and assayed for residual activity as previously described [26]. In this study, an unheated control was considered to be 100% active.

2.2.6. Analysis

The reaction mixtures were preliminarily analyzed using thin-layer chromatography with chloroform–methanol–acetic acid–water (12:6:1:1, v/v) as the developing solvent. The plates were stained with α-naphthol (2.56 g/L) in an ethanol–sulfuric acid mixture (90:10, v/v). The carbohydrates in the samples were observed by heating for a few minutes at 100 °C. (Fig. S3)

Qualitative and quantitative analyzes were carried out using a high-performance liquid chromatograph (HPLC Waters P600) equipped with a refractive index detector. A C18 column (Acchrom, 0.46 cm × 25 cm) was used for analysis at 30 °C with a mobile phase of methanol–H_2O (3:2, v/v), and the flow rate used was 0.7 mL/min. Previously purified and characterized butyl galactopyranoside was used as the standard. The retention time of butyl galactopyranoside was 4.243 min, and for *n*-butanol, it was 5.485 min (Fig. S4).

3. Results and discussion

3.1. Effect of various enzymes

Control experiments were carried out in the absence of microwave irradiation, but with different supported β-galactosidases. The yields markedly varied with the type of β-galactosidase used (Fig. 1). Microwave irradiation did not increase the yields of the reactions catalyzed by TN0949 (β-galactosidase from *T. naphthophila* RKU-10, expressed in our lab) [27], TN0602 (β-glucosidase from *T. naphthophila* RKU-10, expressed in our lab), and AOBG (β-galactosidase from *Aspergillus oryzae*, obtained from Zhongnuo Biotech. Co., Ltd., Jiangsu, China). In contrast, microwave irradiation increased the yields in reactions utilizing TN1577. The differences observed may be attributed to the severe denaturation of TN0949 and AOBG caused by the microwaves. These results indicate that not all enzymes can be activated by microwave irradiation. Based on these findings, TN1577 was considered the best catalyst for synthesizing butyl galactopyranoside; hence, this enzyme was used in all subsequent experiments.