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Enhancing the thermostability of recombinant cyclodextrin glucanotransferase via optimized stabilizer

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ABSTRACT

Cyclodextrin is used widely in industry, and the thermostability of cyclodextrin glucanotransferase (CGTase) is important for high-yield cyclodextrin production. A chemical mixture (25% glycerol, 10% polyethylene glycol 400, and 0.5 mmol/L $CaCl_2$) was used as stabilizer to enhance the thermostability of CGTase after optimizing its concentration, which resulted in a synergistic effect on CGTase stability. The β-CGTase and γ-CGTase activities increased by 7.6- and 9.4-fold, respectively, compared with controls, which resulted from an increase in the energy of inactivation, as determined by a thermokinetic analysis. The optimum temperature of CGTase activity increased from 50 to 60 °C after the stabilizer application. The stabilizer was applied to cyclodextrin production, and it resulted in enhanced CGTase activity, which showed higher increases of starch to CD conversion at 60 and 90 °C. These results demonstrate that using a stabilizer is an effective approach for CGTase storage and cyclodextrin production.

1. Introduction

Cyclodextrins (CDs) attract much attention because they are used in the food, cosmetic, pharmaceutical, and chemical industries, as well as for drug delivery and in agriculture and environmental engineering [1]. CD production through a biocatalytic process using CD glycosyltransferase (CGTase), which is a 1,4-α-D-glucan 4-α-D-(1,4-α-D-glucano)-transferase, is beneficial because it is environmental friendly and easy to operate. For example, commercial CGTase Toruzyme 3.0 L and Amano CGTase catalyze starch to produce produces a mixture of CDs (α, β, γ) from the starch [2]. CGTase catalyzes mainly three kinds of reactions: disproportionation reaction that transfers a linear oligosaccharide chain to another linear oligosaccharide molecule, giving rise to a series of sugar molecules of different sizes, and hydrolytic reaction when water acts as an acceptor, and cyclization which is involved in the cleavage of α-(1,4) linked cyclomaltodextrins (six d-glucose units for α-CD, seven d-glucose units for β-CD, eight d-glucose units for γ-CD) from starch, and intermolecular transglycosylation coupled the resulting maltooligosaccharide to various carbohydrate acceptors [3]. Many processes, such as isoamylase addition in 250-ml Erlenmeyer flasks [4], amylosucrase addition [5], ultrafiltration membrane reactor utilization, [6], and an integrated continuous stirred tank reactor – packed bed reactors process [7], have been explored by researchers for high-yield CD production. During CD production, a major issue is the short working time of CGTases, which is caused by CGTase inactivation [8]. Many CGTases have typical working temperatures in the range of 20–60 °C [3], with optimal temperatures of 50–60 °C, although some CGTases have an optimal temperature of 40 °C [9]. For example, the optimal temperature of *Bacillus circulans* CGTase is 55–60 °C [10], and *Bacillus lehensis* and *Bacillus agaradhaerens* CGTases showed the highest activity at 55 °C [11,12]. Therefore, high thermostability of CGTases is needed to achieve long working times [13]. High CGTase thermostability also has the advantages of low risk of contamination, lower viscosity of starch, and higher starch solubility [14]. And it has been obtained by microbial isolation [15], immobilization to a loofa sponge [16], sodium alginate [17], glutaraldehyde-activated chitosan spheres [18], and gene mutation [19,20]. Chemicals addition is an important approach for enhancing enzyme stability. And polyols, sugars, and salts are effective stabilizers [21]. Generally, hydroxy polyols and sugars engage in strong hydrophobic interactions with enzymes, which results in increased enzyme hydration. Salts have specific and non-specific effects on enzyme thermostability. However, the appropriate stabilizer depends on the nature of the enzyme because stabilizers are highly specific [21]. For example, the half-lives of trypsin and glucose oxidase are increased significantly by the addition of glycol chitosan [22]. Glucose oxidase is stabilized by salts addition [23]. The stability of chloroperoxidase is enhanced by the addition of polyhydroxy addition [24]. The stability of CGTase is enhanced by adding polyethylene glycol or polypropylene glycol for CD production [25]. In addition, enzymes tend to form aggregates with altered properties and that may alter the

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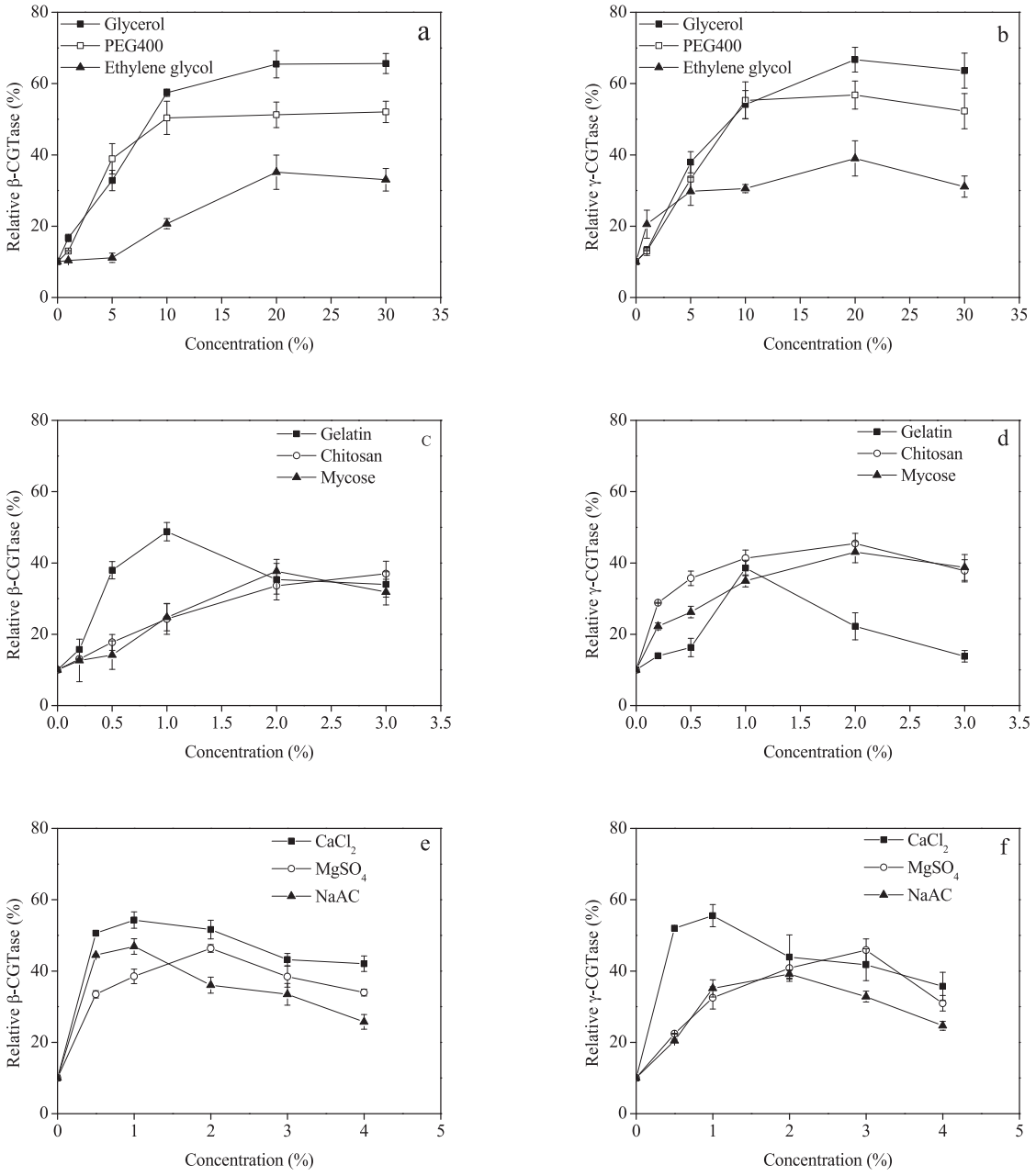

Fig. 1. Effect of chemicals on CGTase activities (a: glycerol, PEG400, Ethylene glycol on β- CGTase activities; b: glycerol, PEG400, Ethylene glycol on γ-CGTase activities; c: gelatin, chitosan, mycose on β-CGTase activities; d: gelatin, chitosan, mycose on γ-CGTase activities; e: $CaCl_2$, $MgSO_4$, NaAC on β- CGTase activities; f: $CaCl_2$, $MgSO_4$, NaAC on γ-CGTase activities).

results of the activity and stability studies [26]. Therefore, the effect of combined stabilizers should be investigated for enzyme stability enhancement. In the present study, chemicals mixture was established to enhance the stability of a recombinant CGTase from *Komagataella phaffii* was enhanced by the addition of a stabilizer. The thermokinetic parameters were analyzed to clarify the changes of stabilizer addition. This recombinant *K. phaffii* harbored a codon-optimized *cgt* gene encoding a CGTase from *Bacillus pseudalcaliphilus* 8SB. This CGTase catalyzed starch to produce β-CD and γ-CD only, without α-CD [27,28]. These results built a base for enzyme stabilizer enhancement by chemicals mixture.

2. Materials and methods

2.1. Materials

Recombinant *K. phaffii* harboring a codon-optimized *cgt* gene encoding a CGTase from *Bacillus pseudalcaliphilus* 8SB [15], according to the Codon Usage Database (http://www.kazusa.or.jp/codon/), was constructed in a previous study for high-level CGTase expression [28]. Only β-CGTase activity and γ-CGTase activities were detected with this CGTase. CGTase ($27.6 \pm 0.7 \times 10^3$ U/mL β-CGT activity, $111 \pm 3 \times 10^{-2}$ g protein/L) was used in this research. Glycerol, $CaCl_2$, $MgSO_4$, and sodium acetate (NaAC) were purchased from the Sino Chemical Company (Shanghai, China). Polyethylene glycol 400 (PEG400), ethylene glycol, gelatin, chitosan, and mycose were purchased from Sangon Biotech (Shanghai, China). Liquid chemicals (glycerol, PEG400, ethylene glycol) solution were prepared using