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Discovery and Characterization of a Novel Method for Effective Improvement of Cyclodextrin Yield and Product Specificity

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Abstract Cyclodextrins(CDs) are widely used in food, pharmaceuticals, drug delivery, and chemical industries and in agriculture and environmental engineering. To improve the yield and selectivity of CDs, this work presented a facile, scalable and efficient enzymatic synthesis of *β*-CD from starch using *β*-cyclodextrin glycosyltransferase (CGTase, EC 2.4.1.19) from *Bacillus cereus.* First, we found that the pretreatment of starch dramatically influenced CDs yield that was related to the structure and molecular weight of the substrate starch. Second, alcohol solvents influenced the yield and product selectivity of CDs; tertiary alcohols enhanced CDs yield(from 54.95% to 68.21%) and secondary alcohols increased the product selectivity(*β*-CD/*γ*-CD changed from 6.25 to 8.05). Fluorescence quenching analysis showed that the binding constants and entropy of the solvents influenced the yield and product selectivity, respectively. In conclusion, the results demonstrate that this study provides a promising method for the industrial production of *β*-CD.

Keywords *β*-Cyclodextrin glycosyltransferase; Alcohol solvent; *Bacillus cereus;* Cyclodextrin

1 Introduction

Cyclodextrins are cyclic oligosaccharides with hydrophilic exterior and hydrophobic internal cavity that offer unique advantages due to the ability to form water-soluble inclusion complexes with numerous poorly soluble lipophilic molecules[1,2]. Therefore, CDs are widely used in pharmaceutical, textile, agricultural, cosmetic, chemical and food industries[3]. CDs are usually produced from starch or starch derivatives by the catalytic action of glycosyltransferase(CGTase). CGTase is an important member of the alpha-amylase family and is an extracellular enzyme that catalyzes the cleavage of the *α*-1,4 linkages in starch or polysaccharides to form CDs[4,5]. The specific mechanisms of cyclodextrin formation have been described by Han *et al*.[6], Van *et al*.[7] and Atanasova *et al*.[8]. CGTase catalyzes four reactions: cyclization, coupling, disproportionation and hydrolysis[9]. In these reactions, cyclization produces CDs, which is the characteristic reaction of CGTase, *i.e*., CGTase catalyzes the *α*-glycosidic bond cleavage of starch and a part of the donor is separated to form CDs as a receptor[10].

CGTase catalyzes the formation of CDs from starch, producing mainly a mixture of *α*-, *β*- and *γ*-CD(with six, seven, and eight glucose units, respectively), thus adversely affecting the separation and purification of CDs[3,6,11,12]. To improve the product specificity of CGTase, the molecular modification of CGTase and the addition of organic solvents can be used. Compared with the molecular modification of CGTase, the addition of organic solvents can increase the product specificity by influencing product inhibition or intermolecular glycosylation and improve the total CDs production[13]. For instance, Tesfai *et al*.[13] found that the recombinant CGTase from *Anaerobranca gottschalkii* produced up to 45% of the total CDs in the presence of ethanol and 91% of *α*-CD and 64% of *β*-CD in the presence of decanol and cyclohexane, respectively. Tomita *et al*.[14] reported that the ethanol increases the yield of *γ*-CD by 2.5-fold. The product specificity of CGTase was influenced by the properties of the organic solvents used: in the absence of any solvent and in the presence of dimethylsulphoxide, tert-butanol and dimethylformamide, *β*-CD was preferentially produced, while *α*-CD was preferentially produced in the presence of acetonitrile, ethanol and tetrahydrofuran[15]. These studies indicated that organic solvents, especially alcohol solvents, have important effects on the yield and product specificity of CDs.

In this context, based on our recently identified *β*-CGTase from *Bacillus cereus*, we examined the biocatalytic activity of the *β*-CGTase in the synthesis of CDs in various alcohol solvents including primary, secondary and tertiary alcohols. The effects of various starch pretreatment methods on CDs production were also examined. Subsequently, the mechanism of the effects of various types of organic solvent on *β*-CGTase was

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predicted by fluorescence quenching.

2 Experimental

2.1 Materials

The alcohol solvents, including ethanol, *n*-butanol, sec-butanol and tert-butanol were purchased from Sinopharm Chemical Reagent Co., Ltd.(Shanghai, China). Tryptone and yeast extract were obtained from Oxoid(Hampshire, UK). The standard samples of α-CD, β-CD, γ-CD and starch were purchased from Sangon(Shanghai, China). All other chemicals used were of analytical grade.

2.2 Production of CDs

Soluble starch(0.3 mol/L) was dissolved in boiled glycine-NaOH buffer(pH=8.5, 50 mmol/L) as the original material. The appropriate amount of the enzyme(800 U/g starch) was added and the reactions were carried out at 55 °C. The details of preparation and purification of recombinant β-CGTase were reported in the Electronic Supplementary Material of this paper (Fig.S1—Fig.S4, Table S1). Samples of the reaction mixture were withdrawn at appropriate intervals and boiled for 10 min to stop the enzymatic reaction.

2.3 High-performance Liquid Chromatography(HPLC) Analysis

The molecular size distribution of the pretreated starch solution was evaluated by HPLC in a CXDH-3000 chromatography work station(Shodex, China) with a Series III pump, refraction index detector(Model Shodex RI-201H), and column heater(Model Timberline HT-130). Separation was achieved by using a sugar column TSKgel G-Oligo-PW 0008031(300 mm×7.8 mm; Tosoh Bioscience) at 60 °C, eluted with water at a flow rate of 0.6 mL/min[16]. The molecular weight of the pretreated starch was characterized based on the molecular weight standard curve of dextran obtained in our laboratory[16,17]. The retention time values of starch pretreated by various methods are shown in Fig.S5(see the Electronic Suplementary Material of this paper) and Table 1.

Table 1 High-performance liquid phase characterization of starch samples obtained by various treatments

Sort	Retention time/min	Solubility/ (mol·L^{-1})	Weight-average molecular weight
Original starch	8.570	0.28	65090
Stirred starch	8.593	0.29	63521
Sonicated starch	8.648	0.29	56071
Sonic broken starch	8.671	0.30	49753
Gelatinized starch	8.739	0.30	46432
Steam treated starch	8.740	0.30	39467

The concentrations of CDs were determined by HPLC using a TSKgel Amide-80 column(250 mm×4.6 mm, Milford, MA) eluted with a mixture of acetonitrile and water(65:35, volume ratio) at 1.0 mL/min and 25 ºC with a refractive index detector. The retention time values of α-CD, β-CD and γ-CD standards were 7.442, 9.437 and 11.207 min, respectively (Fig.S6 and Fig.S7, see the Electronic Supplementary Material of this paper).

2.4 Fluorescence Spectroscopy Measurements

The interaction of β-CGTase with alcohol solvents was studied by using fluorescence spectroscopy. The concentrations of the quenchers(alcohol solvents) were added gradually ranging from 6.25×10^{-9} mol/L to 60.14×10^{-9} mol/L. The fluorescence spectra of the β-CGTase with various types of alcohol solvents in the presence of various concentrations(0, 5.0, 7.5, 10.0 and 12.5 μL/mL, respectively) were measured by a fluorescence spectrophotometer(F-7000, Hitachi, Japan) at 298, 303 and 310 K. The excitation wavelength was set at 300 nm; the emission spectrum was recorded from 200 nm to 800 nm with a scanning speed of 1200 nm/min. The width of the excitation and emission slits was set at 5.0 nm.

The detailed calculation methods of the Stern-Volmer binding constants and thermodynamic parameters are reported in the Electronic Supplementary Material of this paper.

3 Results and Discussion

3.1 Effect of Starch(substrate) Pretreatment on the Yield of CDs

The starch granules contain crystalline and amorphous regions. Raw starch is not easily degraded by β-CGTase because of the compact crystalline structure. To disrupt the crystal structure of the starch, starch degrading enzymes are usually added before β-CGTase to liquefy starch, thus decreasing the CDs yield by consuming a portion of the starch and accelerating the coupling reaction[18]. To find an effective way to treat starch, the effect of starch pretreated by various methods on CDs yield was studied using β-CGTase. CDs were prepared by using the starch solution obtained by pretreatment with various methods. As shown in Fig.1, high temperature and high pressure pretreatment, high temperature gelatinization, ultrasonic crushing pretreatment, ultrasonic pretreatment, and high speed stirring pretreatment can improve the yield of CDs(β-CD and γ-CD) compared to that obtained in the case of the intact starch. The yield of α-CD was too small to be observed consistent with previous reports[19]. After steam treatment or gelatinization, the yield of β-CD was 46.79%, and the yield of γ-CD was 7.58%, both of which were substantially improved compared to original starch(0.14% and 0.02%,

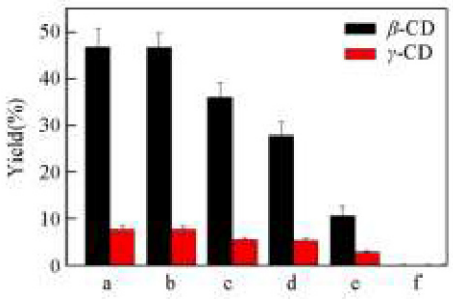

Fig.1 Effect of pretreatment methods on the yield of CDs

The reaction was carried out at 55 °C and pH of 8.5. a. Steam treatment; b. gelatinized; c. sonic broken; d. sonicated; e. stirred; f. control.

respectively).

To explore the reason for the differences in CDs yield caused by various methods of starch treatment, the characterization of starch obtained by various pretreatment methods was performed by HPLC, as shown in Fig.S5 and Table 1. Various pretreatments induce the breaking of the glycosidic chain of the starch, changing the molecular weight of the starch, which is manifested as changes in retention time[16,20]. It should be noted that the weight-average molecular weight of starch decreases concomitant to an increase in the extent of the damage. The weight-average molecular weight of the pretreated starch is the lowest after high-temperature and high-pressure pretreatment reaching 39467. This is consistent with the fact that retention time is delayed concomitant to an increase in the extent of destruction(according to the molecular sieve principle, higher molecular weight corresponds to lower retention time and lower molecular weight corresponds to higher retention time[21]), indicating that the molecular weight was dramatically reduced[16,17]. This phenomenon occurs because as the degree of damage to starch increases, the α-1,4 bond of the starch breaks, and then, the starch with a low molecular weight appears[18]. Moreover, when the weight-average molecular weight of the substrate starch was decreased to a certain value (46432, Table 1), a decrease in the molecular weight of the substrate had little effect on the production of CDs(Fig.1). Thus, when the starch was used as a substrate, the operation of β-CGTase can be effective in the case of starch degradation and reduction in the weight-average molecular weight of starch. Steam treatment and gelatinization are more effective in destroying the crystalline structure of the starch granules, resulting in a substantially higher yield of CDs compared to that of the intact starch because the initial starch has a dense crystalline structure that is not reactive[22]. The natural crystal structure of starch can be destroyed by heating in the presence of water, which irreversibly expands the starch to be many times its original size, producing a large surface area for enzymatic reactions; this phenomenon is known as gelatinization[23]. Additionally, the difference in starch concentrations between the pretreated(0.29—0.30 mol/L) and untreated(0.28 mol/L) samples was small, indicating that pretreatment did not significantly improve the solubility of starch. Then, it was suggested that an increase in CDs production is due to a decrease in the weight-average molecular weight of pretreated starch and the destruction of starch crystal structure, and is not due to the increased solubility of starch.

Therefore, the pretreatment method achieves the purpose of destroying the crystal structure of starch, which can increase the efficiency of the enzyme, and represents an effective way to improve the yield of CDs. The higher the damage to starch is, the more structures of the starch granules open, thus becoming more susceptible to β-CGTase action[18]. Yang *et al.*[24] reported similar results and investigated several starch pretreatment methods. It was concluded that the size of the starch granules and the extent of destruction of the starch crystal structure are important factors influencing the yield of β-CD. As the extent of starch destruction increases, the crystal structure is degraded, the efficiency of the enzyme action is substantially improved, and the CDs yield is improved as a result.

3.2 Effect of Temperature and pH on the CDs Yield

In an enzyme-catalyzed reaction, temperature is an important factor that determines the reaction rate. As show in Fig.2(A), temperature influences the rate of β-CGTase enzymatic reaction by two pathways. First, increasing the temperature(40—55 °C) increases the thermal energy of the substrate molecule and increases the rate of the reaction, thus increasing the yield of β-CD(33.43%—45.44%) and the yield of γ-CD(6.21%—7.52%). However, higher temperatures(>55 °C) have another effect that involves an increase in the molecular thermal energy of the β-CGTase protein structure, which increases the chance of multiple weak noncovalent interactions, such as hydrogen bonding and van der Waals interactions. These interactions maintain the three-dimensional structure of β-CGTase and its disruption may ultimately lead to the unfolding of the enzyme, resulting in a decrease in β-CD(45.44% to 37.20%) and γ-CD(7.52% to 7.03%) yields. The most suitable temperature for CDs production is 55 °C, corresponding to the β-CD and γ-CD yields of 45.44% and 7.52%, respectively [Fig.2(A)], which was close to the optimal temperature of other β-CGTase obtained from similar sources. The optimal bioconversion temperature of *Bacillus circulans* E 192 was found to be 60 °C[25]; Qiu *et al.*[26] reported the maximum yield of γ-CD of 32.9% at 55 °C using the CGTase from *Bacillus licheniformis*; a maximum γ-CD yield reached 45.3% in the case of

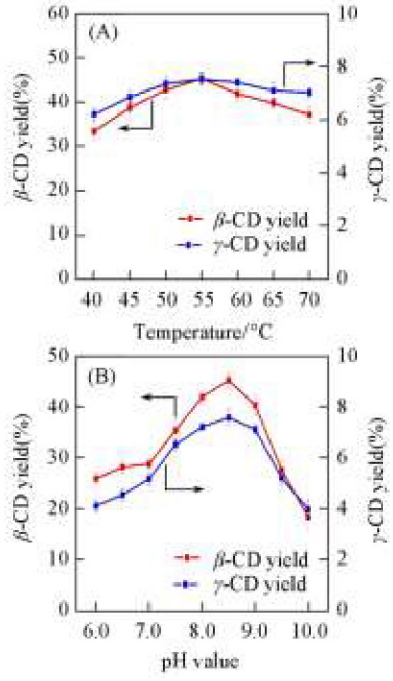

Fig.2 Effects of temperature(A) and pH(B) on the yields of β-CD and γ-CD

(A) The reactions were carried out at different temperatures (40—70 °C) at pH of 8.5, and 800 U/g enzyme solution was incubated with 0.3 mol/L soluble starch solution; (B) the reactions were assayed at different pH(6.0—10.0) at temperature of 55 °C, and 800 U/g enzyme solution was incubated with 0.3 mol/L soluble starch.

CGTase from *Bacillus clarkii* 7364 at the optimal bioconversion temperature of 55 °C[27].

Each enzyme has the most suitable pH and the highest catalytic reaction rate at optimal pH. Fig.2(B) illustrates that the yields of *β*-CD(45.27%) and *γ*-CD(7.58%) are maximal at pH of 8.5. As shown in Fig.2(B), a slight deviation in the optimal pH(8.0—9.0) causes a change in the ionization of a group at the active site of *β*-CGTase and a slight decrease in the yield of *β*-CD(45.27% *vs.* 41.94% and 40.35% at pH of 8.5, 8.0, and 9.0, respectively) and *γ*-CD(7.58% *vs.* 7.21% and 7.11% at pH of 8.5, 8.0, and 9.0, respectively). When the pH shifts are larger (>pH 10.0 and <pH 6.0), numerous covalent bonds that maintain the three-dimensional structure of *β*-CGTase are disrupted, leading to the unfolding of the enzyme[Fig.2(B)]. In the case of recombinant CGTase from *Bacillus licheniformis*, the conversion rate to total CDs reached the highest value(35.5%) at pH of 10.0[26]. The maximum *γ*-CD yield of 41.6% was achieved by using CGTase from *Bacillus clarkii* 7364 at pH of 10.0[27]. The optimal pH values for CDs production by these CGTases are higher than that for CGTases obtained from other organisms, such as *Bacillus firmus*(pH=5.5—9.0)[28] and *Klebsiella pneumoniae* AS-22(pH=6.0—9.0)[29]; these differences are attributed mainly to the nature of the source strains.

3.3 Effect of Various Alcohol Solvents on CDs Yield and Product Selectivity

The reaction process with added organic solvents has been widely used in industrial production to obtain high CDs yield. The methods of CDs production can be classified into two types depending on whether the organic solvents are added in the reaction process[19]. The addition of organic solvents can substantially change the ratio of CDs in the product, thereby increasing the selectivity of the product[15]. The influence of various alcohol solvents on CDs synthesis is shown in Table 2. The alcohol solvents have a higher total CDs yield (62.45%—68.21%) compared to the solvent-free reaction system(54.95%), and the best results were obtained with tert-butanol(68.21%) followed by ethanol and sec-butanol and, finally, *n*-butanol. In general, these alcohol solvents help to dissolve higher amount of the starch substrate thus increasing the yield of total CDs. Comparing the total yield in the case of primary alcohols(ethanol and *n*-butanol) with the yield in the case of secondary(sec-butanol) and tertiary alcohols (tert-butanol) suggests that higher polarity enhances the yield. As shown in Table 2, secondary alcohol has a higher *β*-CD/*γ*-CD ratio(8.05) than that of other alcohol solvents (ethanol, *β*-CD/*γ*-CD=7.69, *n*-butanol, *β*-CD/*γ*-CD=6.98, and tert-butanol, *β*-CD/*γ*-CD=7.67) and solvent-free(6.25) reaction system. This indicates that sec-butanol has a strong influence on the product specificity of *β*-CGTase. Blackwood *et al.*[15] have found that alcohol solvents influence the yield and product specificity of the CDs products; they reported that *β*-CGTase from *Bacillus circulans* strain 251 increased CDs yield in all organic solvents compared with that in a nonorganic solvent and the highest CDs yield of 66% was detected in the presence of ethanol(26%, volume fraction). Moreover, the same *β*-CGTase showed a slight product specificity with regard to the CDs product ratio(5%—10%), while the specificity of the *β*-CD product increased to 82% when tert-butanol was added to the catalytic reaction[15].

Table 2 Effect of solvents on the CDs yield*

Solvent	*β*-CD yield(%)	*γ*-CD yield(%)	CDs yield(%)	*β*-CD/*γ*-CD
None	47.37±0.63	7.58±0.55	54.95±1.15	6.25±0.55
Ethanol	58.95±1.14	7.67±0.37	66.62±2.01	7.69±0.38
n-Butanol	54.62±1.51	7.83±1.34	62.45±2.34	6.98±0.65
sec-Butanol	57.81± 0.87	7.18± 0.93	64.99±1.13	8.05±0.91
tert-Butanol	60.34±1.28	7.87±0.88	68.21±2.12	7.67±0.57

* Values represent the mean±standard deviation of triplicate tests.

3.4 Effects of Alcohol Solvent Concentration on *β*-CD Yield

Organic solvents are widely used in industrial production of CDs to increase the CDs yield[30]. The concentration of an organic solvent in the *β*-CGTase catalytic systems may dramatically influence the enzyme activity and stability. Optimization of the alcohol solvent concentration is shown in Fig.3. The *β*-CGTase produces *β*-CD with a low yield(45.73%) in the absence of alcohol solvents. An increase in the *β*-CD yield was observed in the presence of sec-butanol, tert-butanol, and ethanol(54.93%—61.06%). For instance, the *β*-CD yield increases 1.33-fold for an increase from 0 to 25%. This is due to an increase in the polarity of the reaction system induced by alcohols, thus increasing the solubility of the starch substrates. Structural formulae are frequently useful to discriminate between various groups of the carbon atoms according to their structural characteristics[31]. Classification of alcohol solvents is based on carbon atoms attached to hydroxyl and the polarity and reactivity of the alcohol solvent vary depending on the type of carbon atom attached to hydroxyl[31—34]. This is expected to influence the synthesis of CDs. However, excessive concentration of alcohol solvents can affect the activity and stability of *β*-CGTase. Therefore, when alcohol solvent concentration is higher than 25%, the CDs production does not increase.

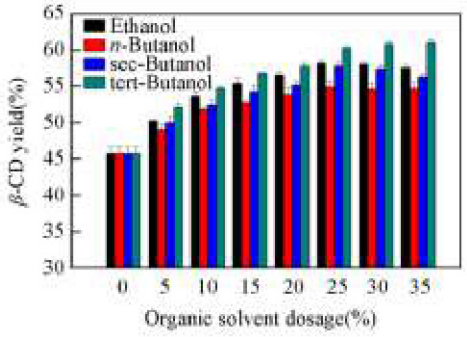

Fig.3 Optimization of the alcohol solvent dosages
The reaction was carried out at 55 °C and pH of 8.5.

3.5 Time Course of Enzymatic Production of *β*-CD

The time course of *β*-CD production catalyzed by *β*-CGTase is shown in Fig.4. It can be seen that the reaction time influences the yield of *β*-CD from 4 h to 44 h, and the

yield reaches 62.27% in 32 h with tert-butanol as the cosolvent; the yield is 1.28 times higher than that in the control (solvent-free) group. However, the production of *β*-CD barely increased when the reaction time exceeded 36 h, suggesting the presence of a stable equilibrium state.

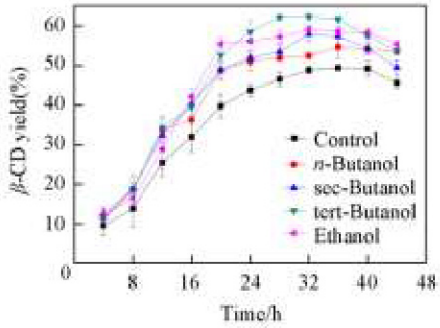

Fig.4 Time course of the *β*-CD yield in alcohol solvent systems

Alcohol solvent(25%, volume fraciton) was added, and the reaction was carried out at 55 °C and pH of 8.5.

Similarly, the *γ*-CD yield increases slowly over time and a maximum yield of approximately 10% was obtained by CGTase from *Bacillus clarkii* 7364 after 21 h in the absence of organic solvents. However, *γ*-CD production increased rapidly during the first 6 h and continued to increase, finally reaching a maximum(*ca*. 31%) after 21 h in the presence of 5-cyclohexen-1-one. The maximal *γ*-CD yield of 32.9% and 86.2% *γ*-CD product ratio to total CDs were achieved at 55 °C and pH of 10.0 in the presence of 5-cyclohexadecen-1-one[26].

3.6 Fluorescence Analysis

To explore the interaction between various types of alcohol solvents and *β*-CGTase, synchronous spectroscopy was used, and the thermodynamic parameters were analyzed. The thermodynamic parameters of the binding reaction can provide substantial evidence for the prediction of the binding[35]. The fluorescence spectra of *β*-CGTase with various types of alcohol solvents are shown in Fig.5. The corresponding values of thermodynamic parameters are shown in Table 3.

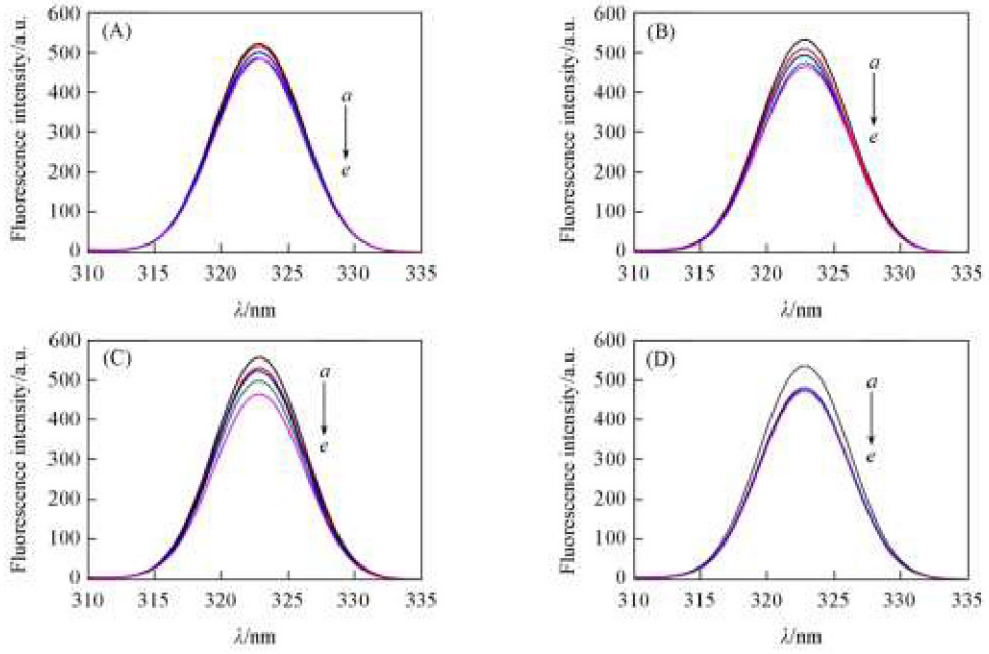

Fig.5 Fluorescence spectra of CGTase with different alcoholic organic solvents

(A) Ethanol; (B) *n*-butanol; (C) sec-butanol; (D) tert-butanol. The excitation wavelength was set at 300 nm, the emission spectrum was chosen from 200 nm to 800 nm with a scanning rate of 1200 nm/min(*T*=298 K, pH=8.5). The width of the excitation and emission slits was set at 5.0 nm. From *a* to *e*, the contents were 0, 5, 7.5, 10, and 12.5 μL/mL, respectively.

According to Fig.5, *β*-CGTase combined with various alcohol solvents has different degrees of fluorescence quenching apparently due to different activities and polarities of each alcohol solvent[34]. The quenching mechanism can be dynamic or static, and these mechanisms can be discriminated by their different dependencies on temperature[35]. Dynamic quenching is caused by collision and static quenching results from the formation of the ground state fluorophore-quencher complex. As a result, the predicted dynamic binding constant increases with increasing temperature and the static binding constant decreases with increasing temperature[35,36]. As shown in Table 3, the binding constants of the four alcohol solvents to *β*-CGTase increase(ethanol: 0.04×10^4—0.09×10^4 mol/L; *n*-butanol: 2.33×10^4—3.50×10^4 mol/L; sec-butanol: 13.16×10^4—44.84×10^4 mol/L; and tert-butanol: 67.10×10^4—94.77×10^4 mol/L) concomitantly with an increase in the temperature from 298 K to 310 K, implying that the interaction between *β*-CGTase and the alcohol solvents increases with increasing temperature and suggesting that the elevated temperature enhances affinity and reaction rate[35,37]. The binding constants of primary alcohol solvents are low(Table 3: ethanol: $K_a=0.04\times10^4$—0.09×10^4 mol/L; and *n*-butanol: $K_a=2.33\times10^4$—3.50×10^4 mol/L), indicating that the affinities are weak. In contrast, tertiary alcohol has a high binding constant (Table 3: tert-butanol: 67.10×10^4—94.77×10^4 mol/L) and a strong affinity. Therefore, the CDs yield in the reaction

system with tert-butyl alcohol as a cosolvent is slightly higher than that in the case of other reaction systems(Table 2).

Table 3 Comparison of Stern-Volmer binding constants K_a and thermodynamic parameters of enzyme-substrate association

Solvent	Temperature/K	10^4K_a/ (mol·L)	ΔH^{θ}/ (kJ·mol^{-1})	ΔS^{θ}/ (J·mol^{-1}·K^{-1})	ΔG^{θ}/ (kJ·mol^{-1})
Ethanol	298	0.04	45.32	203.56	–15.13
	303	0.08			–16.73
	310	0.09			–17.63
n-Butanol	298	2.33	25.92	170.67	–24.91
	303	2.84			–25.83
	310	3.50			–26.97
sec-Butanol	298	13.16	76.13	354.64	–29.21
	303	32.13			–31.94
	310	44.84			–33.54
tert-Butanol	298	67.10	21.61	184.28	–33.24
	303	82.67			–34.32
	310	94.77			–35.47

Fluorescence quenching analysis also revealed different binding modes and stabilizing forces between *β*-CGTase and alcohol solvents. The data of Table 3 demonstrate that all groups have positive ΔS^{θ} and ΔH^{θ} when quenching *β*-CGTase, indicating that hydrophobic interaction is the main force between an alcohol solvent and *β*-CGTase. It should be noted that the ΔS^{θ} value of sec-butanol is substantially higher than that of other groups(354.64 J·mol^{-1}·K^{-1} *vs.* 170.67 J·mol^{-1}·K^{-1} to 188.64 J·mol^{-1}·K^{-1}). The higher value of ΔS^{θ} corresponds to the higher likelihood of the chemical reaction. It is possible that the interaction between *β*-CGTase and sec-butanol is fine-tuning the structure of the enzyme, thus changing the selectivity of the product(Table 2).

4 Conclusions

In summary, biosynthesis using *β*-CGTase from *Bacillus cereus* enzymes, pretreated starch substrate and alcohol cosolvents, resulting in high yield and selectivity. The tertiary alcohol reaction system had a higher CDs yield(from 54.95% to 68.21%), and the secondary alcohol reaction system influenced the product selectivity(*β*-CD/*γ*-CD: from 6.25 to 8.05). Fluorescence quenching analysis predicted that the binding constants and entropy of alcohol solvents influenced the yield and product selectivity, respectively. Moreover, the pretreatment of starch was performed to reduce the weight-average molecular weight of starch to get higher substrate solubility and CDs yield. This biosynthetic pathway has potential value for the industrial production of *β*-CD because it is a straightforward and low-cost reaction system with high selectivity.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s40242-019-8406-0.

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