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Improvement of Steroid Biotransformation with Hydroxypropyl-β-Cyclodextrin Induced Complexation

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Abstract The inclusion complexes induced by cyclodextrins and its derivates have been shown previously to enhance the biotransformation of hydrophobic compounds. Using hydroxypropyl-β-cyclodextrin (HP-β-CD; 20% *w*/*v*), the water solubility of cortisone acetate increased from 0.039 to 7.382 g L^{-1} at 32 °C. The solubilization effect of HP-β-CD was far superior to dimethylformamide (DMF) and ethanol. The dissolution rate also significantly increased in the presence of HP-β-CD. The enzymatic stability of Δ^1-dehydrogenase from *Arthrobacter simplex* TCCC 11037 was not influenced by the increasing concentrations of HP-β-CD contrary to the organic cosolvents which negatively influenced in the order DMF > ethanol. The activity inhibition effect caused by HP-β-CD was not so conspicuous as ethanol and DMF. Inactivation constants of ethanol, DMF, and HP-β-CD were 5.832, 4.541, and 1.216, respectively. The inactivation energy (E_a) was in the order of HP-β-CD (55.1 kJ mol^{-1}) > ethanol (39.9 kJ mol^{-1}) > DMF (37.1 kJ mol^{-1}).

Keywords Hydroxypropyl-β-cyclodextrin · Δ^1-Dehydrogenase · Enzymatic activity · Enzymatic stability · Thermal stability

Abbreviations

CDs	Cyclodextrins
HP-β-CD	Hydroxypropyl-β-cyclodextrin
CA	Cortisone acetate
PA	Prednisone acetate
E_a	Inactivation energy
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
ESI-MS	Electrospray ionization mass spectra

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Introduction

Microbial reaction for the steroid transformation is a typical example of the successful microbial technology application in large-scale industrial process. Specific microbial transformation steps have been incorporated into numerous partial syntheses of new steroids for evaluation as drugs and hormones. Through hydroxylation, side-chain cleavage, dehydrogenation, and miscellaneous reaction catalyzed by microorganism, a variety of steroid drugs were obtained and widely used as anti-inflammatory, diuretic, contraceptive, progestational, anti-androgenic, and anticancer agents [1]. However, the main problem of steroid biotransformation remains its poor water solubility and low dissolution rate, which results in poor availability of substrate. As a result, a lot of efforts have been devoted to improving the solution, including the addition of the water-soluble organic cosolvent [2] or surfactant [3], the use of new organic biocatalysis technology, such as aqueous two-phase system [4], cloud point system [5], pure organic solvent system [6], and ionic liquid system [7]. All of the above methods can enhance the solubility of the substrate. On the other hand, prior studies have also shown that compared with the organic media, the activities of most enzymes are higher in their natural aqueous media [8]. So, the key issue on the microbial transformation of steroids compounds is to improve the solubility and dissolution rate of the substrate and maintain biocatalysts activity and stability.

Dimethylformamide (DMF), dimethyl sulfoxide, and ethanol used as cosolvents are commonly added into the transformation system [9, 10]. These cosolvents not only have the potential to increase the conversion rate by improving the dissolution rate and solubility of steroid compounds but also can alter the enzyme properties by changing its active conformation [11]. Specifically, high concentrations of cosolvent could strip away the essential bound water from the surface of enzyme and finally lead to the deactivation of enzyme. Therefore, the effects of cosolvents should be investigated for the biotransformation process to establish the most compatible solvent and its addition amount.

Cyclodextrins (CDs) are known to form inclusion complexes with many types of organic molecules for their intermolecular hydrophobic cone-like cavity. They have received considerable attention in pharmaceutical application because of their improvement in water solubility, chemical stability, and bioavailability of various drugs through the inclusion complexes formation [12]. Besides, CDs not only have a good biocompatibility with microorganisms but also have a benefaction to respiratory-chain activity [13]. This useful combination prompts their use in microbial transformations of hydrophobic compounds [14]. HP-β-CD is a common and typical kind of β-CD derivates. It maintains β-CD's inclusion complex property and improves the solubility of β-CD at the same time. With a relatively low price, HP-β-CD has a broad prospect in practical application.

In our previous research, HP-β-CD, DMF, and ethanol were selected from many organic additives for improving steroid 1-en-dehydrogenation process. In order to choose a proper additive that could improve the availability of substrates with the minimum lost in enzyme activity and stability, the changes in the steroid solubility and dissolution rate resulted from the addition of HP-β-CD, DMF, and ethanol were first tested. The pH alteration analysis followed. Effects of additives on enzymatic stability, activity, and thermal stability were investigated systematically. Furthermore, the ratio of the host and guest molecule in complexes was preliminary certified by the electrospray ionization mass spectra analysis (ESI-MS).

Materials and Methods

Materials

Cortisone acetate (CA) was supplied by Tianjin Pharmaceutical Company (99.4% purity). Standard CA and prednisone acetate (PA) were purchased from Sigma-Aldrich Co. and HP-β-CD was from Xi'an Deli Biology & Chemical Industry Co. Ltd. (31.7% degree of substitution, 1,523 average relative molecular mass). Other salts and solvents were of analytic grade from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (China).

Microorganism Cultivation and Preparation of Whole *Arthrobacter simplex* TCCC 11037 Cells

A. simplex TCCC 11037 stored in our laboratory was maintained at 32 °C on slant consisting of glucose 10 g L^{-1}, yeast extract 10 g L^{-1}, and agar 20 g L^{-1} (pH 7.5). Seed medium consisted of glucose 10 g L^{-1}, corn slurry 10 g L^{-1}, peptone 5 g L^{-1}, and KH_2PO_4 2.5 g L^{-1} (pH 7.2). The medium used for *A. simplex* TCCC 11037 cells cultivation was the same as seed medium. *A. simplex* TCCC 11037 cells were prepared in two consecutive cultivation steps (18 h for seed culture and 27 h for cell cultivation, respectively). At 32 °C, the whole *A. simplex* TCCC 11037 cells grew in 500-mL shake flasks containing 100 mL culture media using 5% (*v*/*v*) of seed culture on a rotary shaker (160 rpm). A 0.01% (*m*/*v*) CA was used as an inductor of *A. simplex* TCCC 11037 Δ^1-dehydrogenase after 16 h growth. The cells were centrifuged at 4,500×*g* for 15 min, washed with KH_2PO_4-NaOH buffer twice (0.05 M, pH 7.2). The buffers used in this experiment were all the same.

Steroid Transformation and Analysis

Buffers (30 mL) were prepared with the same additive concentration (5% (*m*/*v*)) and the same CA concentration (3% (*m*/*v*)). They were preincubated in 250-mL shake flask at 32 °C, 180 rpm for 10 min to keep temperature equilibration. Equivalent washed cells were added (2.00 mg dry weight mL^{-1}). Samples (500 μL) were drawn at various time intervals, while continuously being shaken. The samples were extracted by chloroform and dried in vacuum, then the solid extracts were redissolved in eluent (dichloromethane/ether/methanol, 86:12:2 (*v*/*v*)) and filtered through a 0.2-μm filter. They were assayed by high performance liquid chromatography (HPLC; Agilent 1100, USA), measuring absorbance at 240 nm. Analysis was performed on a Kromasil 100-5SIL, 250×4.6-mm column with a flow rate of 0.8 mL min^{-1}. The concentration of CA and PA was determined from calibration curve, making from eluent solutions of standard CA and PA.

Solubility and Dissolution Rate Measurements of CA

An excess of CA was added to 30-mL additive-containing buffer of varying concentrations (0–20% *w*/*v*). Samples were incubated under the condition identical to those employed for bioconversion (32 °C, 180 rpm). The flasks were tightly sealed to avoid evaporation. After 24 h, 1-mL aliquot of the slurry was withdrawn and filtered through a 0.2-μm filter. The filtrate was dried in vacuum and redissolved in eluent and then analyzed by HPLC. To

investigate the influence of HP-β-CD on CA dissolution rate, in 250-mL shake flask, 30 mL phosphate buffer containing 5% (*w*/*v*) of HP-β-CD was added, using 30 mL buffer without HP-β-CD as control. After preincubating at 32 °C to keep temperature equilibration, excess CA was added; 0.5 mL slurry was withdrawn every 10 min and filtered through a 0.2-μm filter. The filtrate was also dried in vacuum and redissolved in eluent and then analyzed by HPLC.

Effect of Additives on pH

In 25 mL glass bottle, the relevant additives were added at various concentrations to a final volume of 10 mL. The solutions were shaken for 10 min (32 °C 160 rpm), and the final pH values were measured using a Metrohm 744 pH meter.

Enzymatic Stability Analysis

Buffers containing different concentrations of relevant additives were prepared. After resuspending the equivalent washed cells in prepared buffers, the suspensions were incubated at 32 °C for 10 min. Subsequently, the buffers containing different additives were removed by centrifugation. After washing the cells twice, they were resuspended in pure buffer. The remaining enzymatic activity was determined by preincubating for 10 min at 32 °C to keep temperature equilibration, followed by the addition of CA to a final concentration of 0.5 g L^{-1}. Samples (500 μL) were drawn at various intervals, while being continuously shaken (180 rpm), and then analyzed by HPLC.

Enzymatic Activity Analysis

Buffers (30 mL) containing different concentrations of relevant additives were prepared, which contained the same concentration of CA 0.038 g L^{-1} (below its saturation solubility at 32 °C). Equivalent washed cells were resuspended and the enzymatic activity was assayed as described in "Enzymatic Stability Analysis" section.

Thermal Stability Analysis

Buffers containing 10% (*w*/*v*) of the relevant additives were prepared. Equivalent cells were resuspended into the prepared buffers and incubated at various temperatures from 10 °C to 50 °C in a shaking water bath. At specific time intervals, 5-mL suspension was withdrawn and the remaining enzymatic activity was assayed immediately as described in "Enzymatic Stability Analysis" section.

ESI-MS Analysis of Complexes

ESI-MS analysis was performed on an ion trap mass spectrometer in positive ion model (LCQ Advantage Max, Thermo-Finnigan, USA), which equipped with an ESI source. The sheath gas rate was 10 arb and ion spray voltage was 4.5 kV in full scan model. Five percent (*m*/*v*) HP-β-CD was added in 10% (*m*/*v*) methanol buffer solution at room temperature with the identical CA concentration in "Enzymatic Activity Analysis" section. The methanol was added to prevent CA precipitation at room temperature. Samples filtered through a 0.2-μm filter and analyzed by ESI-MS.

Results and Discussion

Effect of Additives on CA 1-en-Dehydrogenation

CA conversion curves in the presence of additives (5% *m*/*v*) were shown in Fig. 1. The use of HP-β-CD facilitated CA biotransformation process conspicuously. CA conversion ratios in the presence of HP-β-CD were higher than 50% after 3.5 h and higher than 90% after 9 h biotransformation, while in ethanol- and DMF-containing buffer, 7 and 8 h were needed for the 50% conversion ratio. The final CA conversion ratio in the presence of HP-β-CD reached 97%, which was obviously higher than that in ethanol- (92%) and DMF (88%)-containing buffer. Although the final CA conversion ratios in ethanol- and DMF-containing buffer could reach 95% or higher at relative low initial CA concentrations (i.e., 0.5% *m*/*v*, date not shown), the low final conversion ratio in this experiment might be caused by the ethanol and DMF's limited solubilization effect (shown in "Solubility and Dissolution Rate of CA" section). The hydrophobic product could crystallize using undissolved CA particles as crystal seeds and encapsulated them, which could prevent cells from accessing and digesting the undissolved CA. Furthermore, the Δ^1-dehydrogenase denaturation resulted from ethanol and DMF (shown in "Effect of Additives on Enzymatic Stability" and "Effect of Additives on Enzymatic Activity" sections) should not be ignored.

Solubility and Dissolution Rate of CA

Since the steroids solubility and dissolution rate are the limiting steps for biotransformation process [15], the solubilities of CA with various concentrations of additives were determined at the reaction temperature of 32 °C. According to Fig. 2, the solubilization effect was in the order: HP-β-CD » DMF > ethanol. A linear increase in solubility with increasing additive concentrations was observed for each additive. Though both DMF and ethanol improved the solubility of CA, the most significant increase in solubilization was observed for HP-β-CD. The apparent solubility of CA increased as a function of HP-β-CD concentration. Based on the shape of phase-solubility curve generated by HP-β-CD, it could be preliminarily classified to A-type curve, which was consistent with Jadoun and

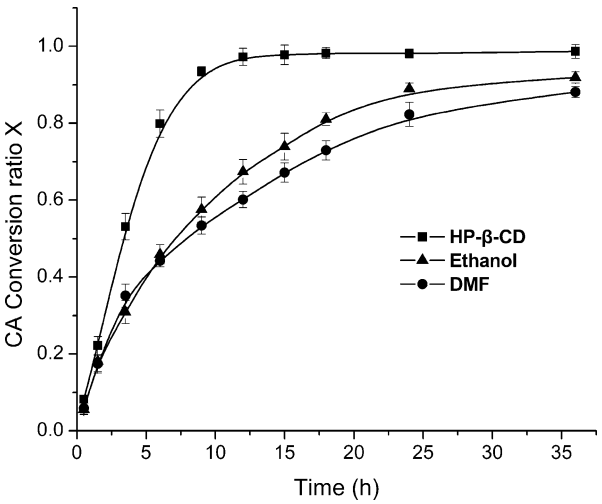

Fig. 1 Effect of additives on CA 1-en-dehydrogenation process

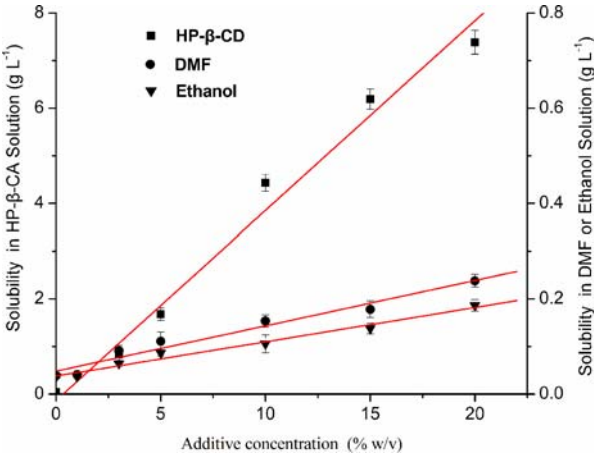

Fig. 2 Effect of three additives on CA solubility in phosphate buffer

Bar's research [16]. The solubilization effects for each additive were at the same level when the additive concentration was at 1% (*m*/*v*). When the additive concentration increased to 3% or higher, the solubilization effect for HP-β-CD was more significant than that of DMF and ethanol. At 3% HP-β-CD, CA (0.831 g L^{-1}) was almost 12-folds as soluble in 3% ethanol (0.068 g L^{-1}) and 9.1-folds as soluble in 3% DMF (0.091 g L^{-1}). At 20% HP-β-CD, 7.382 g L^{-1} were observed, which are 37-folds and 31-folds of the solubilization effect for ethanol and DMF. It was almost 190 times of the solubility in pure buffer at 32 °C.

Since the kinetics of dissolution is dependent on the stirring and temperature conditions, the dissolution rate measurement was performed under the conditions identical to those of the microbial transformation. The dissolution rate (g L^{-1} min^{-1}) was expressed by the specific value between the saturated solubility and the time for reaching the saturated solubility. The CA dissolution rate in the presence of 5% (*w*/*v*) HP-β-CD was 0.168 g L^{-1} min^{-1}, while in the presence of HP-β-CD, the CA dissolution rate was 0.325×10^{-3}g L^{-1} min^{-1}. Singer and Shity also demonstrated β-CD accelerated androstenedione dissolution rate [17]. Therefore, the addition of HP-β-CD promoted the dissolution rate and the solubility of CA significantly, which could improve the biotransformation reaction rate.

Effect of Additives on pH

The influence of additives on the pH of the reaction medium is an important variable for the enzymatic reaction. Previous research had shown that the optimal pH for steroid Δ^1-dehydrogenase from *A. simplex* was 10.0 [18]. Concentrations ranging from 5% to 50% (*w*/*v*) were investigated for the three additives: ethanol, DMF, and HP-β-CD. The results were shown in Table 1. Although the regular definition of pH could not be applied directly in this study since the activity coefficient of the hydrogen ions in solutions containing organic

Table 1 Effect of additives on the pH of 0.05 mol L^{-1} phosphate buffer with an initial pH of 7.2.

Additive concentration (% *w*/*v*)	5	10	20	30	40	50
DMF	0.15	0.32	0.67	1.08	1.76	2.30
Ethanol	0.15	0.29	0.61	0.90	1.34	1.42
HP-β-CD	0.03	0.05	0.11	0.14	0.19	0.22

solvents may not be the same as their coefficients in solvent-free water, good reproducibility was obtained. The values of increased pH were compared well to the data presented by Yeates and Krieg [19].

The introduction of addictive led to the increase in the pH of the buffer. The presence of DMF and ethanol resulted in a significant increase with the increasing addictive concentration. This change could be attributed to the fact that many kinds of organic cosolvent had Lewis acid and base properties. Cosolvent containing oxygen or nitrogen in their structure generally conferred basic properties to the solution [20]. In contrast to DMF and ethanol, HP-β-CD had a slight effect on the pH of the phosphate buffer even the maximal value of increased pH was less than 0.22. The dates also demonstrated that when using organic cosolvent (i.e., DMF or ethanol), the final pH should be monitored. Due to the negligible effect on pH, HP-β-CD was comparatively a proper additive taking the role as cosolvent.

Effect of Additives on Enzymatic Stability

The effect of various additive concentrations on the enzymatic stability was expressed by the initial conversion rate (R_a) obtained through additive incubation relative to the initial conversion rate (R_0) achieved when no additive was used during incubation. The initial conversion rate was estimated from the slope of conversion process curve when CA conversion ratio was not higher than 30%. According to Fig. 3, after 10 min incubation, the inactivation effect of three additives was in the order DMF > ethanol » HP-β-CD. The increasing concentrations of DMF and ethanol brought about a linear inactivation of the enzyme, while HP-β-CD had a negligible effect on the stability lost of the Δ^1-dehydrognase. Interestingly, the relative reaction rate was more than 1.0. It indicated that there was a slight improvement of the *A. simplex* TCCC 11037 cell's catalytic activity after being incubated in HP-β-CD solution. This could be ascribed to the interaction between CDs and cell membrane or cell wall by virtue of either their complexing ability and/or surface activity as mentioned by Donova and Nikolayeva [21]. Thus, HP-β-CD would be an excellent additive to retain the enzyme stability than DMF or ethanol in phosphate buffer.

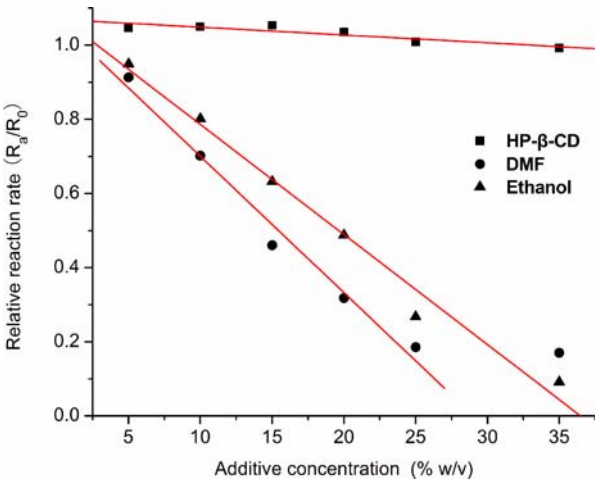

Fig. 3 Effect of three additives on the enzymatic stability of Δ^1-dehydrogenase

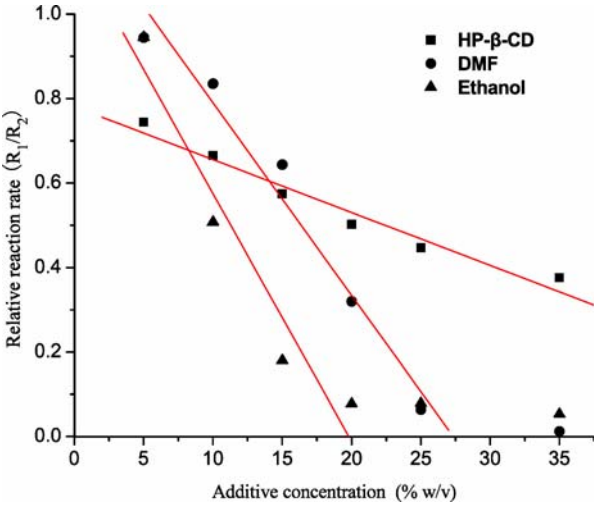

Fig. 4 Effect of the three additives on the enzymatic activity of Δ^1-dehydrogenase

Effect of Additives on Enzymatic Activity

The addition of organic compounds would induce the changes in protein structure and the disruption of noncovalent forces [21]. So, the key issue when cosolvent added is to diminish the lost of enzymatic activity to the maximum extent. To ensure that only the effects of the additives on the enzyme were investigated, an initial substance concentration of 0.038 mg mL^{-1} was applied, which was lower than the saturated solubility of CA at 32 °C. The effect of various additive concentrations on the enzymatic activity was expressed by the initial conversion rate (R_1) achieved with each concentration of the different additives relative to the initial conversion rate (R_2) achieved without any additive. The initial reaction rate was obtained as described in the above section.

According to Fig. 4, each additive led to an inhibition in initial conversion rate which was directly proportional to the additive concentration. This inhibitory effect followed the order: HP-β-CD < ethanol < DMF by calculating the slope of the tendency line. As mentioned by Nellaiah and Morisseau [22], the inhibition of an enzyme subjected to an additive could be quantified by calculating either the additive concentration leading to a 50% decrease in initial reaction rate called half-inhibitory concentration or by calculating the inactivation constant from the slope of the straight lines. These values were also calculated and listed in Table 2.

According to the above results obtained for DMF and ethanol, increased solubility comes at the cost of decreased activity. Although HP-β-CD had the minimal inactivation constant (1.216 *v*/*w*) and the highest half-inhibitory concentration (22.333% *w*/*v*), an

Table 2 Half-inhibitory concentrations and inactivation constants of Δ^1-dehydrogenase from *A. simplex* TCCC 11037 for three additives.

Additive	Half-inhibitory concentration (%, *w*/*v*)	Inactivation constant (*v*/*w*)
HP-β-CD	22.333	1.216
DMF	16.556	4.541
Ethanol	11.379	5.832

obvious decrease in relative reaction rate was observed for HP-β-CD. It was likely that HP-β-CD decreased the enzymatic activity which was contradictory to the result shown in Fig. 1. Nevertheless, compared with HP-β-CD content (5–35% (*w*/*v*) corresponded to 0.033–0.230 mol L^{-1}) in this experiment, we must take account of the extremely low CA content (0.038 mg mL^{-1} corresponded to 0.095×10^{-3} mol L^{-1}). With the increase of HP-β-CD concentration, the equilibrium of Eq. 1 shifted to the right side and the 1:1 and 2:1 inclusion compounds contents increased while free CA content decreased. HP-β-CD's hydrophilic surface could restrict the access of CA to microorganism. So, the decrease of enzymatic activity could be attributed to the decreased permeation of CA across the cell membrane.

$$[\mathrm{HP}-\beta-\mathrm{CD}]+\mathrm{CA} \leftrightarrow ([\mathrm{HP}-\beta-\mathrm{CD}]-\mathrm{CA}) \overset{\mathrm{HP}-\beta-\mathrm{CD}}{\longleftrightarrow} ([\mathrm{HP}-\beta-\mathrm{CD}]-\mathrm{CA}-[\mathrm{HP}-\beta-\mathrm{CD}]) \quad (1)$$

However, HP-β-CD could act as a reservoir for accessible substrate when substrate concentration was much higher than its saturated solubility at 32 °C (i.e., 30 g L^{-1} equivalent to that in Fig. 1). The exchange rate of the CA between its free and complexed form was faster than the CA dissolution rate. Therefore, the free CA could be kept at a relative higher concentration in conversion process. That could be the reason why HP-β-CD accelerated reaction rate.

The molar ratio of HP-β-CD and CA in inclusion complex was further investigated by ESI-MS. Illustrating from Fig. 5, both 1:1 and 2:1 inclusion complex were observed.

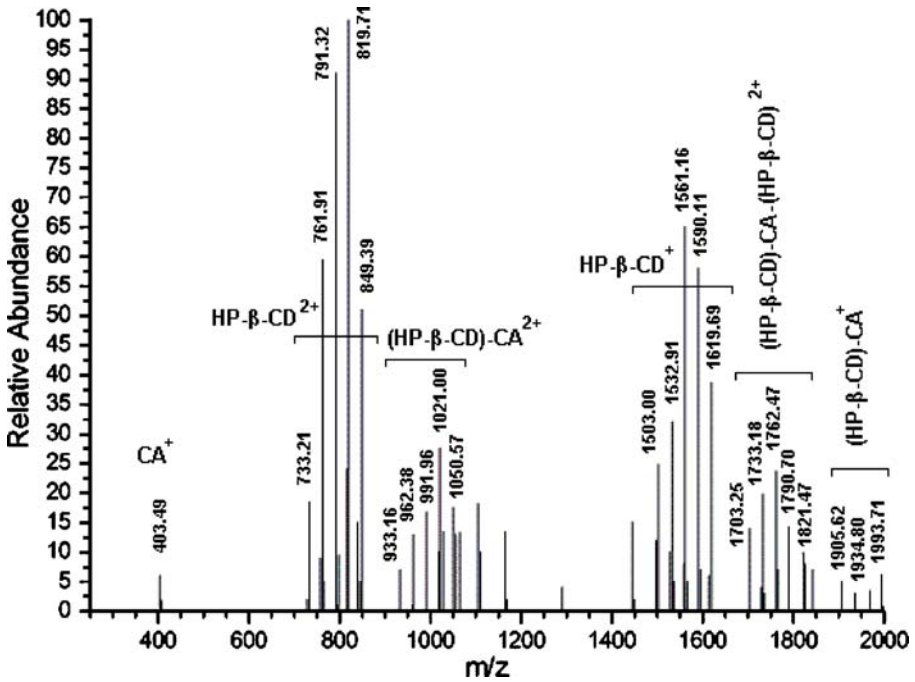

Fig. 5 Full scan electrospray mass spectra obtained for inclusion complex formed by HP-β-CD and CA

Although free CA was also observed, the content was extremely low compared with complexed CA and HP-β-CD. This agreed with the explanation for the decrease of enzymatic activity. CDs could complex with CA molecule on both sides and form the 2:1 inclusion complex as mentioned by Renata and Vianna [23]. Taking the influence of HP-β-CD on *A. simplex* TCCC 11037 Δ^1-dehydrogenase stability (Fig. 3) into account, it was shown that the observed decrease of enzymatic activity in the presence of HP-β-CD was not due to the denaturation of the Δ^1-dehydrogenase, but rather due to the reversible inclusion complex formed with the substrate.

Effect of Additives on Enzymatic Thermal Stability

Generally, the thermal stability of protein depends on their specific amino acid sequence. High temperatures induce an irreversible deactivation of enzymes. Meanwhile, the low temperature is good for keeping the enzyme stability. Pervious research had shown that epoxide hydrolase from *Arthrobacter niger* followed linear deactivation with the passage of time at initial stage, and first order deactivation kinetics were supposed [24]. To establish the effects of the three additives on the thermal stability of the *A. simplex* TCCC 11037 Δ^1-dehydrogenase, the cell suspensions were incubated at various temperatures for different times in the presence of 10% (*w*/*v*) of each additive. The remaining activity as a function of incubation time yielded linear tends and indicated first order deactivation kinetics. Therefore, the inactivation constants (k) for each of the additive at various temperatures were obtained from the slopes of tendency lines.

A usual assay measure of temperature-induced inactivation was the half-life ($t_{1/2}$) of the enzyme, which was defined as the time needed to decrease the initial enzyme activity by 50%. The half-lives in the presence of additives were calculated from the respective inactivation constants (k) by Eq. 2 and illustrated in Fig. 6.

$$t_{1/2} = 0.693/k \tag{2}$$

Although the results showed an exponential decrease of the thermal stability in HP-β-CD-containing buffer, the values of $t_{1/2}$ were much larger than that in DMF- or ethanol-containing buffer. Half-life ($t_{1/2}$) of *A. simplex* TCCC 11037 Δ^1-dehydrogenase in the

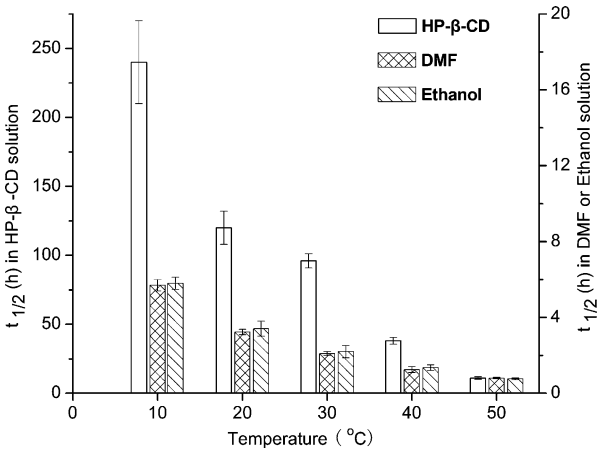

Fig. 6 Half-lives obtained with 10% of additives at various temperatures

presence of HP-β-CD expressed about 60-folds, 13-folds higher than that in the presence of DMF at 10 °C and 50 °C, respectively. This could be attributed to the toxicity of the organic solvents on the protein. Meanwhile, the $t_{1/2}$ for each additive decreased with the increase of temperature could be explained by the increased contribution of temperature induced denaturation at higher temperatures. DMF and ethanol had the similar detrimental influence on enzyme thermal stability; comparatively, ethanol had a relatively slight effect on it. That was coincided with the result that the effect of DMF on enzymatic stability was slightly larger than that of ethanol at 32 °C after 10 min incubation (shown in Fig. 3).

According to the Arrhenius' law Eq. 3

$$\ln k = \ln A - \frac{E_a}{RT} \tag{3}$$

where A is a constant, E_a is the activation energy of deactivation or thermal inactivation energy (kJ mol^{-1}), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T is the absolute temperature (K).

Plotting ln$k$ as a function of $1/T$, a line was obtained of each additive in Fig. 7.

$$\text{slope} = \frac{E_a}{R}$$

The inactivation energy in the presence of 10% (w/v) of each additive was calculated by the multiplication of slope and R, and the established E_a values obtained for HP-β-CD DMF and ethanol were 55.1, 37.1, and 39.9 kJ mol^{-1}, respectively. The smaller values of E_a and $t_{1/2}$ (at various temperatures) obtained for DMF and ethanol indicated a relatively low thermal stability, which further affirmed the advantage of HP-β-CD. The deactivation order was DMF> ethanol> HP-β-CD.

Conclusion

As is shown above, HP-β-CD is an effective solubilizer to increase the solubility and dissolution rate of steroid in water phase during the biotransformation process catalyzed by

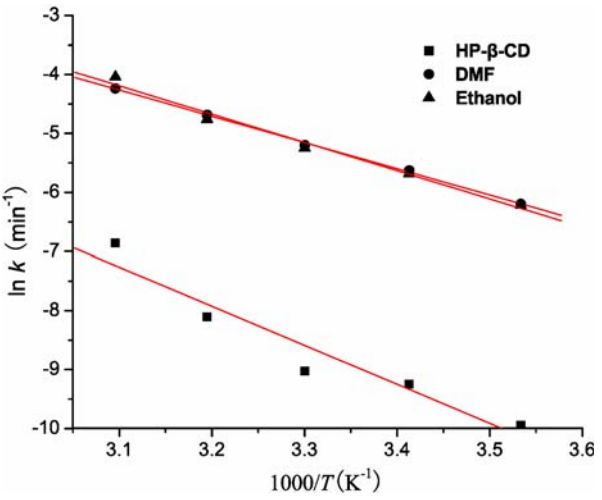

Fig. 7 Arrhenius plot of ln$k$ vs. $1/T$ in the presence of 10% (w/v) HP-β-CD, ethanol, and DMF

enzymes. Much less HP-β-CD is needed to attain a specific solubility, so the activity inhibition caused by additive could be declined to the maximum extent. Both enzymatic stability and thermal stability are least affected by HP-β-CD compared with DMF and ethanol. Although the addition of HP-β-CD results in a decrease in enzymatic activity, the effect of activity inhibition is much less than that with equal quantity of DMF and ethanol; besides, we also assume that this activity inhibition is not caused by the denaturation of the Δ^1-dehydrogenase. Therefore, HP-β-CD not only successfully improves the solubility of the substrate but also minimizes the activity lost and stability lost of the biocatalysts. It is also shown that HP-β-CD had the least affection on pH, so no pH monitoring and adjustment is required, resulting in a simplification of application steps. Furthermore, HP-β-CD is nontoxic and inert to microorganism contrary to organic cosolvent; it has a bright prospect of application to replace organic solvent in biocatalysis and biotransformation fields. This work may not only provide sufficient proofs for HP-β-CD advantages but also provide a convenient and powerful method for efficiently enhancing enzyme-catalyzed steroids biotransformation.

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