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Enhancement of steroid hydroxylation yield from dehydroepiandrosterone by cyclodextrin complexation technique

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ABSTRACT

The cyclodextrins (CDs) complexation technique was performed for the enhancement of hydroxylation yield from dehydroepiandrosterone (DHEA) by *Colletotrichum lini* ST-1. Using DHEA/methyl-β-cyclodextrin (M-β-CD) or DHEA/hydroxypropyl-β-cyclodextrin (HP-β-CD) inclusion complexes as substrate (10 g/L), the hydroxylation yields were increased by 14.98% and 20.54% respectively, and the biotransformation course was shortened by 12 h. X-ray diffractometry, differential scanning calorimetry, and phase solubility analyses showed an inclusion complex was formed between CDs and DHEA at a molar ratio of 1:1, which remarkably increased the solubility of DHEA, and then improved substrate biotransformation efficiency and hydroxylation yield. Meanwhile, results of thermodynamic parameters (ΔG, ΔH, ΔS and *Ks*) analysis revealed the complexation process was spontaneous and DHEA/CDs inclusion complex was stable. Scanning electron microscopy and transmission electron microscopy showed that the enhancement of DHEA hydroxylation yield also depended on the improvement of cell permeability through interaction between cytomembrane and CDs. These results suggested that the CDs complexation technique was a promising method to enhance steroids hydroxylation yield by increasing steroids solubility and decreasing membrane resistance of substrate and product during biotransformation process.

1. Introduction

Dehydroepiandrosterone (DHEA) and its derivatives are important steroid intermediates for many hormone pharmaceuticals. The significant bioactivity of product 7α-OH-DHEA has been proven to up-regulate immunity and prevent the hypoxic cell death of neurons in vitro [1,2]. While 7α,15α-diOH-DHEA is a key intermediate in the synthesis of aldosterone antagonists, such as the new oral contraceptive Yasmin (6β,7β,15β,16β-dimethylene-3-oxo-17α-pregn-4-ene-17,21-carbolactone) [3]. Steroid biotransformation is attracting considerable attention because of its potential commercial value and role as a valuable alternative to traditional chemical processes. According to the previous report, several strains such as *Mucor racemosus* and *Fusarium moniliforme* could transform DHEA into 7α-hydroxy-dehydroepiandrosterone (7α-OH-DHEA) or 7β-hydroxy-dehydroepiandrosterone (7β-OH-DHEA) [4,5]. Also, *Fusarium oxysporum* was described for its ability of hydroxylation in the 15α-position of DHEA [6]. However, reports on the biotechnological aspects of double hydroxylation of DHEA in the C7α- and C15α-positions are limited.

Nowadays, strains that could dihydroxylate DHEA are mainly derived from genera *Colletotrichum*, *Gibberella*, *Fusarium* and *Nigrospora* [3]. Among them, *Colletotrichum lini* was the strain with the highest potential; it showed a hydroxylation yield of 5.6 g/L–5.8 g/L under a repeated batch mode and a final substrate concentration of 7 g/L [7]. Similar to other steroid biotransformation, the remarkable hydrophobic properties and low transferring rates of steroids into and out of the cell were the main limiting factors of DHEA biotransformation [8–10]. In order to solve these problems, methods such as adding organic solvent or surfactant, and applying an organic–aqueous biphasic system have been developed to improve the solubility and bioavailability of substrates. However, the toxicity of organic solvents harmed the activity and stability of biocatalysts and consequently hindered their application on steroids biotransformation [11].

Abbreviations: DHEA, dehydroepiandrosterone; 7α-OH-DHEA, 7α-hydroxy-dehydroepiandrosterone; 7β-OH-DHEA, 7β-hydroxy-dehydroepiandrosterone; 7α,15α-diOH-DHEA, 7α,15α-dihydroxy-dehydroepiandrosterone; CDs, cyclodextrins; M-β-CD, methyl-β-cyclodextrin; HP-β-CD, hydroxypropyl-β-cyclodextrin; XRD, X-ray diffractometry; DSC, differential scanning calorimetry.

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Cyclodextrins (CDs), a homologous group of cyclic glucans consisting of α-1,4-bound glucose units, could form inclusion complexes with a variety of hydrophobic substrates. Gedeon Richter Ltd. (Budapest, Hungary) firstly used macrocyclic α-, β-, and γ-CD for the transformation of steroids solubilized in aqueous media in 1981 [12]. Since then, CDs have received considerable attention for its application in steroid transformation process [13–17]. However, no report on the use of CDs for the hydroxylation of DHEA has been documented.

In this study, a CDs complexation approach was applied for converting DHEA into 7α-OH-DHEA and 7α,15α-diOH-DHEA by *C. lini* ST-1. To evaluate the effects of CDs on DHEA hydroxylation, the substrate conversion efficiency, biotransformation course and the corresponding hydroxylation yield were examined. In addition, the characteristics of the DHEA/CDs inclusion complexes and effects of CDs on cytomembrane permeability were also investigated for exploring the improvement of DHEA hydroxylation yield with the addition of CDs. These findings were of significant importance for steroids biotransformation and could be employed for biotransformation of other homologous substrates with poor solubility.

2. Materials and methods

2.1. Materials

DHEA, 7α,15α-diOH-DHEA were supplied by Tianjin Pharmaceutical Company (99.5% of purity). 7α-OH-DHEA (98% of purity) was prepared in our laboratory through the previous reported method [4]. Methyl β-cyclodextrin (M-β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) were obtained from Zhiyuan Biotechnology Co., Ltd (Shangdong, China). All steroid compounds and other chemicals were of analytical grade and obtained from commercial sources.

2.2. Microorganism and cultivation

C. lini ST-1 was isolated from a factory district in Tianjin, China and deposited in the China General Microbiological Culture Collection Center (CGMCC 6051; Beijing, China). *C. lini* ST-1 was maintained at 30 °C on slant consisting of the following composition (g/L): glucose 30, $FeSO_4$ 0.01, $NaNO_3$ 3, K_2HPO_4 1, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, and agar 20. The strain *C. lini* ST-1 was grown on a rotary shaker (200 rpm) at 30 °C in flasks (500 mL) with 100 mL seed medium of the following composition (g/L): glucose 15, yeast extract 15, soybean cake powder 10, KCl 1, $(NH_4)_2HPO_4$ 1, K_2HPO_4 1, and $MgSO_4 \cdot 7H_2O$ 1. The medium was adjusted to pH 7.0 with 0.5 M NaOH or HCl solution.

2.3. Transformation of DHEA

After 24 h of growth in seed medium, the culture was inoculated with 10% volume into fresh transformation medium (30 mL) containing the following components (g/L): glucose 15, yeast extract 15, corn steep liquor 3, KCl 1, $(NH_4)_2HPO_4$ 1, K_2HPO_4 1, and $MgSO_4 \cdot 7H_2O$ 1. The medium was adjusted to pH 7.0 with 0.5 M NaOH or HCl solution. Then, the fine powder of DHEA or DHEA/CD inclusion complex was added into the medium after cultivating for another 24 h. Transformation reaction was carried out on a rotary shaker (220 rpm) at 28 °C for 60–72 h. Samples were then withdrawn at regular intervals and the products were subsequently extracted by ethyl acetate and dried in vacuum.

2.4. Analysis of transformation products

Analysis of DHEA transformation products were carried out by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

TLC was performed using a 0.25 mm thick layer of silica gel G (Qingdao Marine Chemical Inc., Qingdao, China) in the system of chloroform/methanol (15:1) and visualized by spraying the plates with sulfuric acid/ethanol (1:1), and heated at 60–80 °C for 5 min.

The samples used for HPLC (Dionex, USA) were redissolved in acetonitrile/water (7:3) and filtered through a 0.22 μm microfiltration membrane. Analysis was performed on the Agilent C18 column (5.0 μm, 4.6 mm × 250 mm) at the wavelength of 206 nm, column temperature of 30 °C, with acetonitrile and water (7:3) as the mobile phase at a flow rate of 0.5 mL/min.

2.5. Preparation of inclusion complex and physical mixture

The inclusion complexes between DHEA and M-β-CD or HP-β-CD were prepared by freeze-drying (Labconco, MO, USA) according to the methods reported by Williams [18]. Different molar ratios of DHEA and cyclodextrin were added together as solid and then dispersed in aqueous solution, mixing at a room temperature for 60 h until complexation achieved an equilibrium state. The suspension was filtered through a 0.45 μm microfiltration membrane, and then the filtrate was frozen at −80 °C and lyophilized in a freeze-dryer.

The physical mixture consisting of DHEA and M-β-CD or HP-β-CD at the same molar ratio as the inclusion complex was admixed together in a mortar with pestle for 5 min to obtain a homogeneous physical mixture.

2.6. X-ray diffractometry

X-ray diffractometry (XRD) patterns were recorded on a BRUKER D8 Advance diffractometer (Bruker AXS, Germany) system with CuKα radiaton (λ = 0.15406 nm), over a range of 2θ angles from 3° to 60°. The measurement conditions were as follows: target, Cu; filter, Ni; power, 1600 W (40 kV × 40 mA); scanning rate, 4°/min; angular step, 0.010°.

2.7. Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis of DHEA, CDs, as well as their physical mixtures and inclusion complexes were performed with a HHT-3320A thermal analyzer (Huihetang Biotechnology Ltd., Shanghai, China) from 30 °C–370 °C at a heating rate of 10 °C/min with argon as the carrier gas. The samples were accurately weighed 20 mg and heated in a sealed aluminum pans. Duplicated determinations were performed for each sample. The temperature and heat flow were calibrated using standard tin samples.

2.8. Phase solubility studies

Solubility studies were performed according to the method of Higuchi and Connors [19]. Different concentrations of M-β-CD or HP-β-CD and an excess of DHEA were added to 30 mL aqueous solution in 250 mL shake flasks, then the shake flasks were operated at 28 °C, 220 rpm. After 24 h of equilibration, the suspensions were filtrated through a 0.45 μm microfiltration membrane; and the DHEA concentrations in the filtrate were assayed by HPLC. The apparent stability constant (*Ks*) of the DHEA/M-β-CD or DHEA/HP-β-CD complex was calculated from the phase solubility diagram according to the equation below:

$$Ks = \frac{slope}{S_0(1 - slope)} \quad (1)$$

where *Ks* is the stability constant of the 1:1 complex; S_0 is the intrinsic solubility of DHEA in the absence of CDs; slope is the

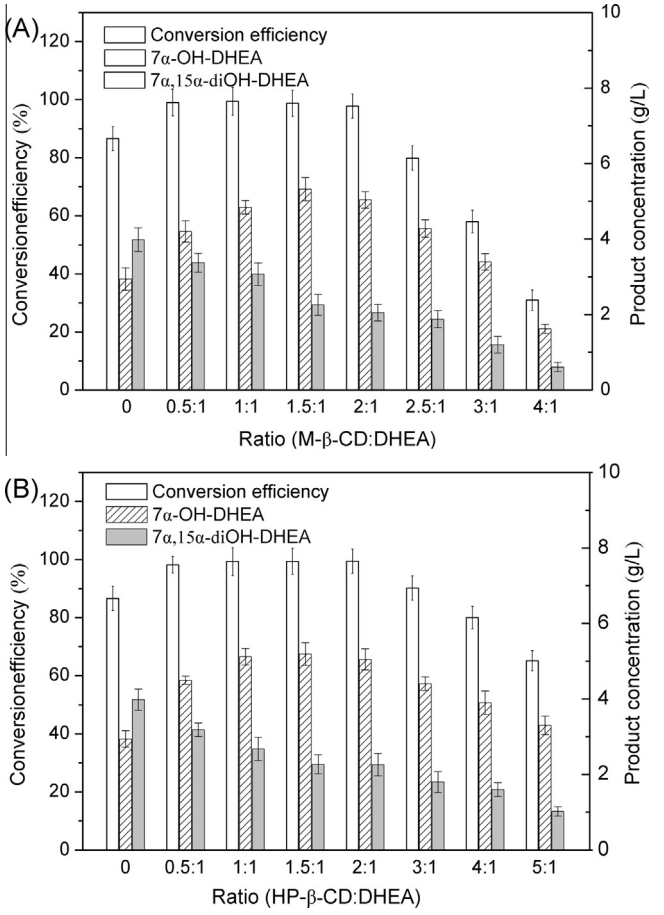

Fig. 1. Effect of the molar ratio of (A) M-β-CD and (B) HP-β-CD to substrate on DHEA conversion efficiency.

saturation concentration of DHEA measured without CDs (intercept).

The stability constants (*Ks*) at different temperatures were used to determine different thermodynamic parameters. The entropy (ΔS) of complexation was calculated using the Van't Hoff equation as follows:

$$\ln(K) = \Delta S/R - \Delta H/RT \quad (2)$$

A plot of $\ln(K)$ versus $1/T$ indicate that the intercept is the value of $\Delta S/R$. The free energy changes (ΔG) and enthalpies (ΔH) of the complexation between DHEA and CDs were determined using the following equations:

$$\Delta G = -RT \ln K \quad (3)$$

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

2.9. Effects of CDs on cells permeability

The surface morphology of the *C. lini* ST-1 incubated with CDs was determined by scanning electron microscope (SEM) and transmission electron microscopy (TEM).

3. Results and discussion

3.1. DHEA biotransformation by CDs complexation technique

3.1.1. Effects of molar ratio (CDs:DHEA) on DHEA conversion efficiency

CDs has been widely used in steroids biotransformation to improve the substrate aqueous solubility, due to their hydrophilic

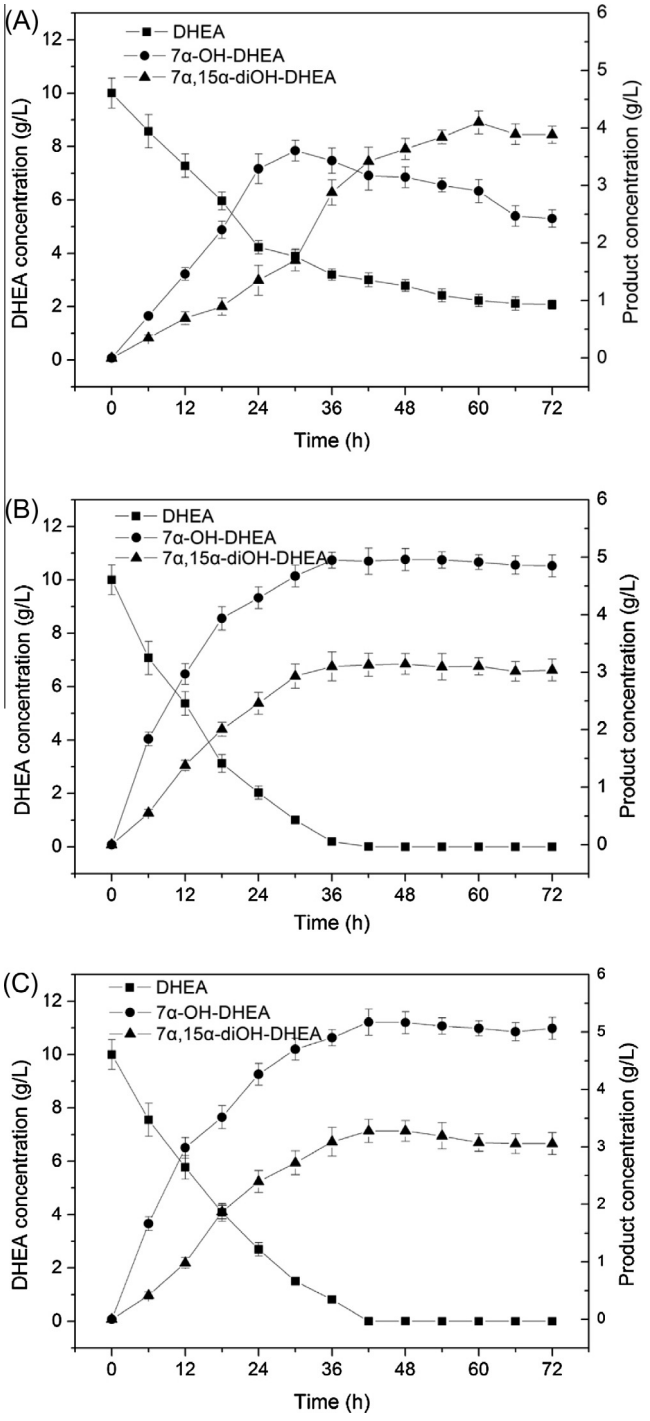

Fig. 2. Conversion process of DHEA with M-β-CD (A) and HP-β-CD (B).

outer surfaces and a hydrophobic cavity at the center [13,15,19,20]. Among the six CDs investigated (α-CD, β-CD, γ-CD, CM-β-CD, M-β-CD, and HP-β-CD), M-β-CD and HP-β-CD were found to be the most effective at improving DHEA hydroxylation production in *C. lini* ST-1 biotransformation (data not shown). Therefore, M-β-CD and HP-β-CD with different concentrations were chosen and applied for DHEA hydroxylation.

As seen in Fig. 1A and B, it was quite evident that the conversion efficiency and hydroxylation yield (7α-OH-DHEA and 7α,15α-diOH-DHEA) increased with the increasing CD concentrations in some extent. However opposite results were found when the CD to DHEA ratio in the media was more than 2:1. The data suggested

that a high CD concentration was toxic to *C. lini* ST-1 hydroxylase and might prevent or delay the proceeding of hydroxylation. In addition, at higher concentration of CD, DHEA molecular was enclosed by CD and "less accessible" for biotransformation, which limited the conversion efficiency in return. At the optimum molar ratio of 1:1, the substrate conversion efficiency and product accumulation were 99.35% and 7.79 g/L with M-β-CD as well as 99.45% and 7.90 g/L with HP-β-CD respectively. By contrast, the control samples without CDs achieved only 86.64% and 6.92 g/L.

3.1.2. Effects of CDs on hydroxylation process

To monitor the changes of substrate and product concentrations during bioconversion process, the conversion samples with or without CDs were withdrawn at regular intervals and assayed by HPLC.

The dynamics of the accumulation of hydroxy product showed in Fig. 2A led to an assumption that the formation of 7α,15α-diOH-DHEA from DHEA occurs at the subsequent introduction of hydroxyl groups in the 7α-position and then in the 15α-position of the steroid molecule [3]. Moreover, the yields of 7α-OH-DHEA and 7α,15α-diOH-DHEA accumulated constantly with the substrate DHEA consuming. The highest yields of hydroxylation products (7α-OH-DHEA and 7α,15α-diOH-DHEA) were 8.06 g/L with M-β-CD and 8.45 g/L with HP-β-CD (Fig. 2B and C), which were enhanced by 14.98% and 20.54% compared with the control (7.01 g/L, Fig. 2A). Meanwhile, conversion course of the maximum product

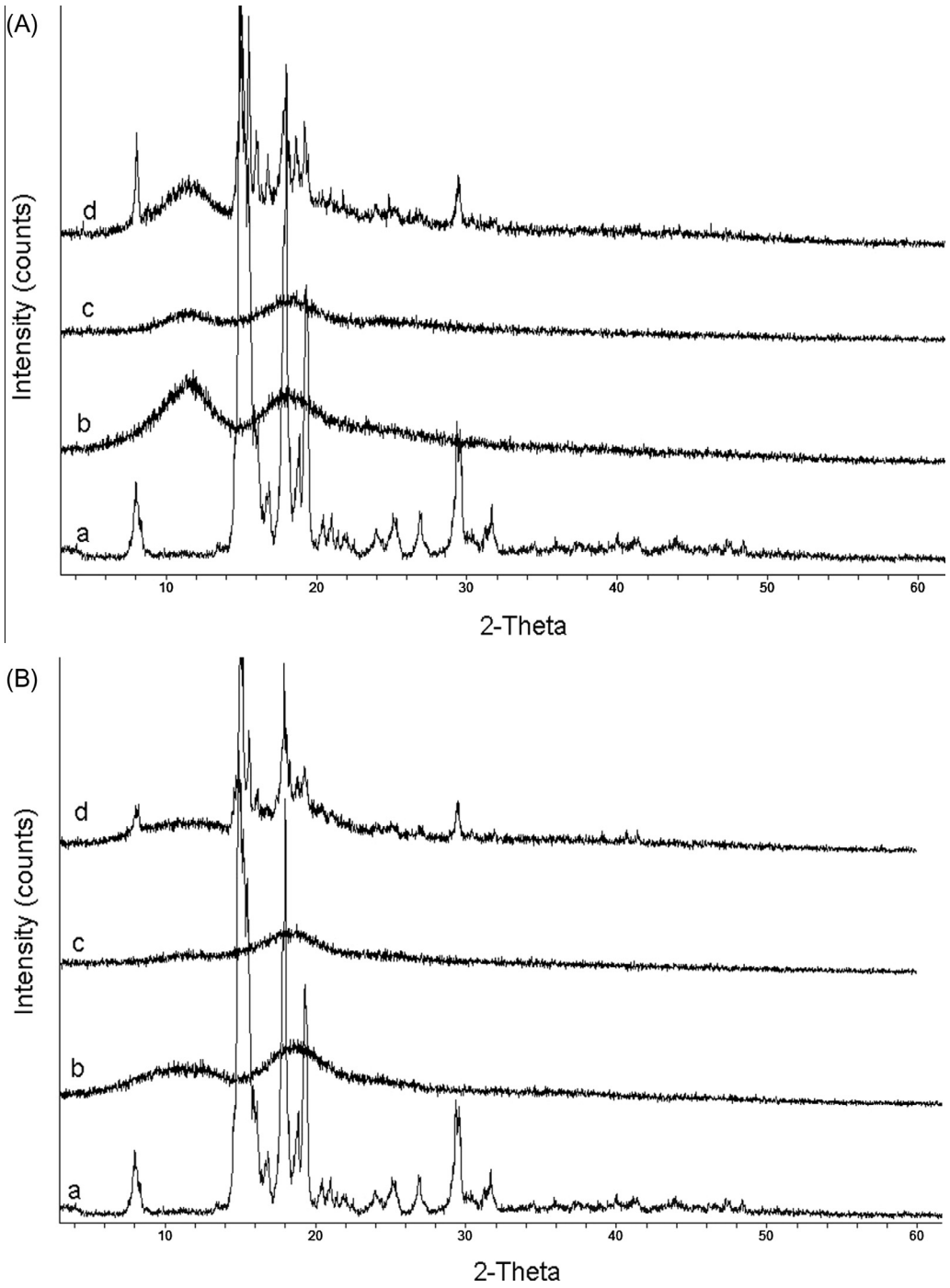

Fig. 3. (A) X-ray diffractograms of (a) DHEA, (b) M-β-CD, (c) inclusion complex, and (d) physical mixture. (B) X-ray diffractograms of (a) DHEA, (b) HP-β-CD, (c) inclusion complex, and (d) physical mixture.

accumulation was shortened from 60 h to 48 h comparing to the process absence of CDs.

Interestingly, the proportion of the two biotransformation products was changed in the presence of CDs. As shown in Fig. 2B and C, the rate of converting DHEA to 7α-OH-DHEA was increased substantially in preliminary 30 h with the addition of CDs. And the 7α-OH-DHEA yield was enhanced by 37.12% with M-β-CD and 43.21% with HP-β-CD comparing to the absence of CDs. However, the rate of converting 7α-OH-DHEA to 7α,15α-diOH-DHEA in presence of CDs was increased slightly and similar to the rate in the absence of CDs after 18 h. The first hydroxylation product of 7α-OH-DHEA in the presence of CDs was not converted into 7α,15α-diOH-DHEA in the latter transformation period, and the yield of 7α,15α-diOH-DHEA was decreased by 23.41% with M-β-CD and 20.24% with HP-β-CD. These results suggested that the presence of CDs suppressed the transformation of 7α-OH-DHEA to 7α,15α-diOH-DHEA in some extent, and a single target product would be achieved by regulating the molar ratio of DHEA to CDs.

In order to illustrate the effects of CDs on DHEA biotransformation elaborately, two aspects including CDs solubilized steroids and CDs affected cell structure of *C. lini* ST-1 were investigated in the following experiments.

3.2. Characterization of the CDs/DHEA inclusion complex

3.2.1. X-ray powder diffractometry

X-ray technique is a useful method to characterize the formation of an inclusion complex between steroid and CDs. In this study, the XRD patterns of M-β-CD, DHEA, DHEA/M-β-CD inclusion complex and their physical mixtures were investigated (Fig. 3A). In addition, the diffraction patterns of HP-β-CD, DHEA, DHEA/HP-β-CD inclusion complex and their physical mixtures were also determined (Fig. 3B).

The X-ray pattern of DHEA (Fig. 3A-a and B-a) exhibited numerous sharp peaks that indicated its crystalline nature. On the other hand, the X-ray pattern of M-β-CD (Fig. 3A-b) and HP-β-CD (Fig. 3B-b) showed no crystalline peak, which suggested their amorphous nature. The diffractograms of physical mixture (Figs. 3A-d and Fig. 3B-d) were superimposed figures of DHEA and M-β-CD or HP-β-CD but with lower intensity compare with each pure components. This was due to the dilution of DHEA in physical mixtures [18]. Meanwhile, X-ray spectrum of the two inclusion complexes (Fig. 3A-c and B-c) showed no characteristic diffraction peaks of DHEA, which was distinct from the corresponding physical mixtures. This difference indicated the lack of crystallinity for the physical mixture and it might provide evidence for the formation of an inclusion complex between DHEA and CDs. Similar results were found by Wang et al. [20] and Forgo et al. [14].

3.2.2. Differential scanning calorimetry

The DSC analysis of DHEA, CDs, their physical mixtures and inclusion complexes were performed (Fig. 4A and B). The DSC thermogram of DHEA (Fig. 4A-a and B-a) demonstrated a sharp endothermic peak at about 150 °C, which was consistent with its melting point. According to previous studies, the melting point of pure DHEA was proved to be 150.9 °C in literature [21]. The DSC curve of M-β-CD (Fig. 4A-b) and HP-β-CD (Fig. 4B-b) displayed a broad endothermic peak between 50 °C to 125 °C and 75 °C to 150 °C, respectively, with no characteristic thermal signal. A similar result was also reported for characterization of the inclusion complex of 16,17α-epoxyprogesterone with M-β-CD [22], which was attributed to the release of water molecular from the cavity. Both the endothermic peak of DHEA and CDs were observed in the physical mixture curve (Fig. 4A-c and B-c). However, the sharp endothermic peak of DHEA disappeared in the inclusion complex system in the present study (Fig. 4A-d and B-d) and the curve

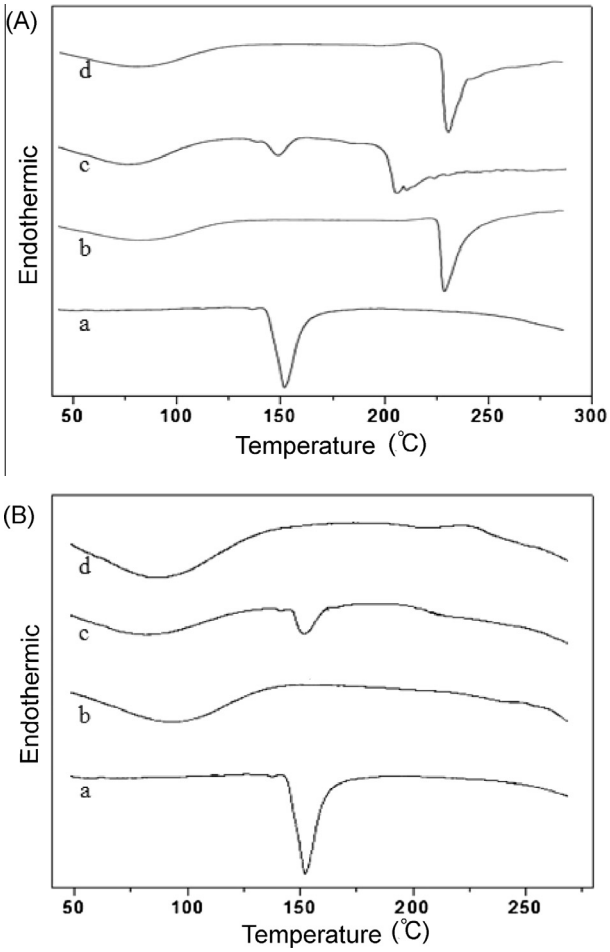

Fig. 4. (A) DSC thermograms of (a) DHEA, (b) MCD, (c) complex mixture, and (d) inclusion complex. (B) DSC thermograms of (a) DHEA, (b) HPCD, (c) complex mixture, and (d) inclusion complex.

was identical to the thermogram of CDs. The difference between inclusion complex and physical mixture indirectly indicated that the product obtained by the freeze drying method maybe not simply a physical mixture but the CDs/DHEA inclusion complex. This finding agreed with the XRD analysis results.

3.2.3. Phase solubility study

Phase solubility study was carried out by gently shaking the aqueous containing an excess amount of DHEA and M-β-CD or HP-β-CD at 0 mM to 60 mM concentration (Fig. 5). The solubility

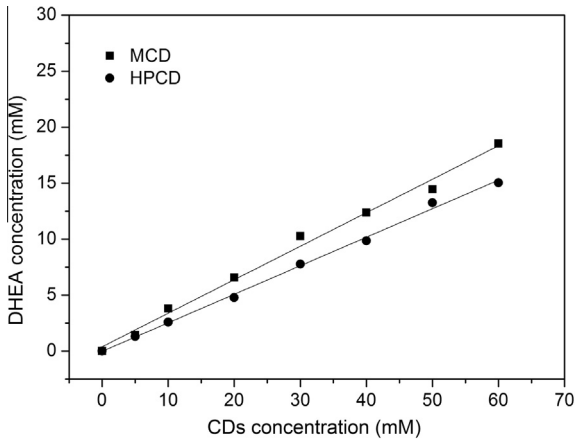

Fig. 5. Phase solubility diagram of DHEA with CDs in aqueous solution at 30 °C.

of DHEA linearly increased with increasing CDs concentration. The plot showed a typical A_L-type solubility curve classified by Higuchi and Connors [19], indicating that the complex of DHEA and CDs was formed at a molar ratio of 1:1. The inherent maximum solubility of DHEA in aqueous was 0.015 mM, and the maximum solubility increased to 18.54 mM and 15.05 mM with the addition of 60 mM M-β-CD and HP-β-CD, respectively. The apparent *K*s values of the DHEA/M-β-CD and DHEA/HP-β-CD complex were calculated to be 28989 M^{-1} and 20284 M^{-1}, which suggested the feasibility of generating DHEA/CDs complexes. Moreover, DHEA/M-β-CD complex was relatively more stable than DHEA/HP-β-CD complex when comparing their *K*s values. This result could attribute to the steric hindrance caused by the presence of hydroxypropyl substituent. Similar results were also observed in the characterization of the inclusion complex of 16,17α-epoxyprogesterone with M-β-CD and HP-β-CD [22].

3.2.4. Thermal analysis

The effects of temperature on the solubilizing ability of CDs in aqueous were studied (Fig. 6A and B) and relevant thermodynamic parameters (ΔH, ΔS, and ΔG) of complexation were reported in Table 1. Although the solubility of DHEA showed marked improvement with the increasing temperature, the apparent *K*s value decreased surprisingly. This was similar to the results observed by Cerchiara [23] and Badawy [24]. It was deduced that the reduction of the interaction forces, such as Van der Waals, hydrogen bonding force and hydrophobic interaction between the guest molecule and CDs were probably the main causes [22,25]. The negative enthalpy demonstrated that the complexation of DHEA with M-β-CD or HP-β-CD was exothermic due to the water molecule in the inner CDs cavity was substituted by DHEA, and the energy release supported the formation of an inclusion complex. Furthermore, the negative value of the Gibbs energy indicated that the formation process of inclusion was spontaneous, whereas the entropy changes suggested that the hydrophobic effects was a strong driving force for DHEA/M-β-CD or DHEA/HP-β-CD inclusion action [22]. The complexation process of inclusion complex formed between DHEA and CDs was spontaneous and stable through the thermal analysis.

3.3. Effect of CDs on cell structure

Cell permeability is another important factor limiting the steroid bioconversion process, besides the solubility of steroid substrates. As previous literature reported, the permeability of microbial cells could be affected by CDs [26]. Therefore, the changes in cell surface structure (cell walls and cytoplasmic membrane) of *C. lini* ST-1 were examined by SEM and TEM, after cultivating of the microorganism with and without CDs for 12 h.

The scanning electron micrographs showed that the control cells had distinct outlines and smooth surface (Fig. 7A). Similarity, the TEM observations showed that the control cells were surrounded by the cell membranes with compact surface, without release of intracellular components and notable ruptures on cell surface (Fig. 8A and B). Compared with the untreated control, remarkable modifications of cell wall and cell membranes were found after exposure to CDs for 12 h. As shown in Fig 7B and C, the cells treated with M-β-CD or HP-β-CD appeared rather rough with numerous unknown components making the cell outline uneven by the observation of SEM. The mycelial morphology and outermost cell surface changed, but no destructive alteration in cell integrity was observed.

The TEM data displayed that the cell walls of *C. lini* ST-1 cultivated with M-β-CD loosened and apparently thickened, whereas the membranes became folded and inconsecutive (Fig. 8C and D). The cell also showed reduced electron density, and some ruptures that could cause slight leakages of cellular cytoplasmic contents

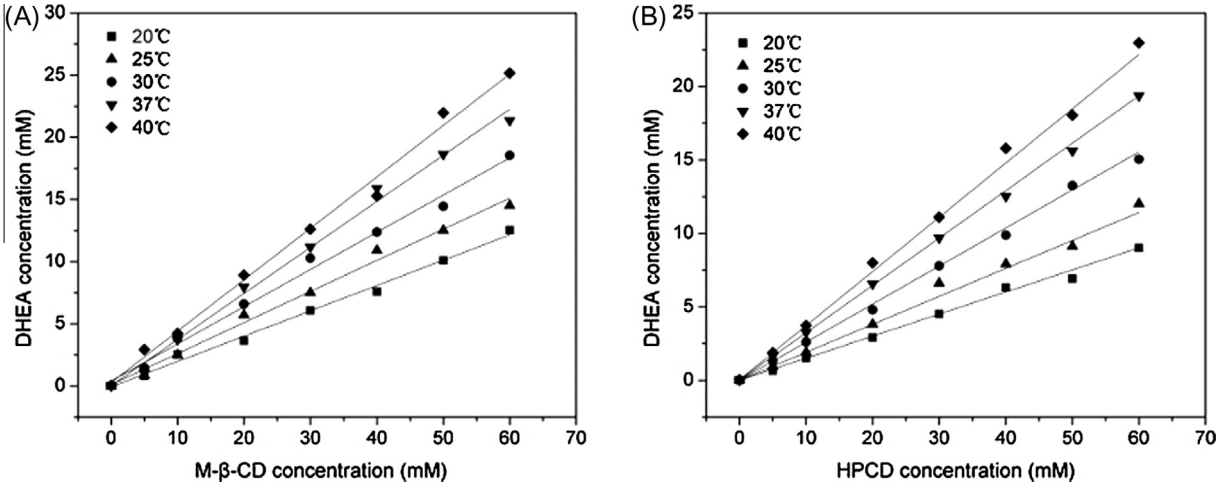

Fig. 6. Phase solubility diagrams for DHEA with (A) M-β-CD and (B) HP-β-CD at different temperatures.

Table 1
Thermodynamic parameters of the inclusion process of DHEA in M-β-CD or HP-β-CD.

T (K)	DHEA/M-β-CD complex				DHEA/HP-β-CD complex			
	$K_{1:1}$ (M^{-1})	ΔG (kJ M^{-1})	ΔH (kJ M^{-1})	ΔS (JMK^{-1})	$K_{1:1}$ (M^{-1})	ΔG (kJ M^{-1})	ΔH (kJ M^{-1})	ΔS (J$M^{-1}K^{-1}$)
293	40442.2	−25.841	−21.78		27.95	−24.94	−20.98	
298	34144.5	−25.86	−21.73		23.745	−24.96	−20.93	
303	28988.9	−25.88	−21.68	13.87	20.28	−24.99	−20.89	13.52
310	23257.3	−25.92	−21.61		16.40	−25.02	−20.82	
313	21226.0	−25.93	−21.59		15.02	−25.03	−20.79	

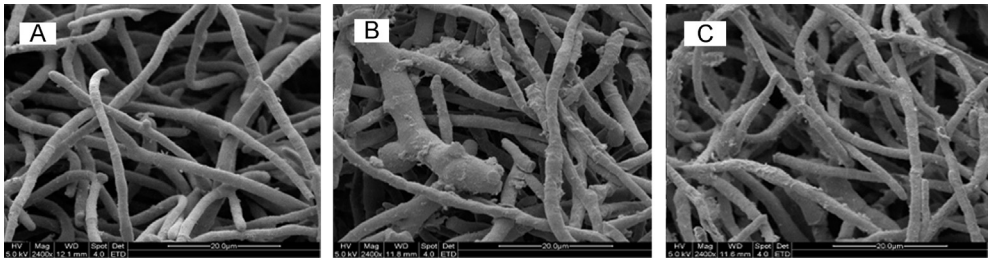

Fig. 7. Effect of CDs on cells structure of *C. lini* by SEM: (A) cells grown without CD (control); (B) cells grown with M-β-CD; (C) cells grown with HP-β-CD.

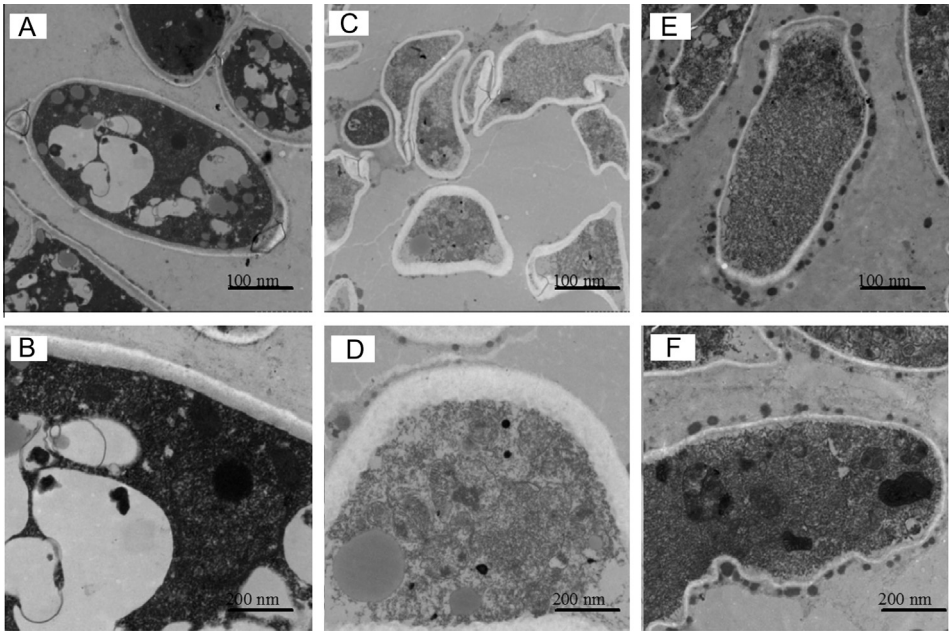

Fig. 8. Effect of CDs on cells structure of *C. lini* by TEM: (A and B) cells grown without CD (control), (C and D) cells grown with M-β-CD and (E and F) cells grown with HP-β-CD.

were also observed on the cell membranes of *C. lini* ST-1. By contrast, the effects of HP-β-CD on the cell surface structure were weaker than those of M-β-CD at the same concentration. As shown in Fig. 8E and F, although the cell walls damaged by the addition of HP-β-CD became thinner, the cell membrane appeared integrated and showed no visible leakages of cytoplasmic contents.

According to these findings, we assumed that the cultivation of *C. lini* ST-1 in the medium with M-β-CD or HP-β-CD affected the cell walls and membranes in some extent. This might be beneficial to increase cell permeability as well as facilitate the absorption and subsequent transport of the steroid substrate from the cell wall to underlying membrane structure.

4. Conclusion

The culture of *C. lini* ST-1 was used for hydroxylating of DHEA, combining with CDs complexation method. The hydroxylation yields were enhanced to 7.79 g/L with M-β-CD and 7.90 g/L with HP-β-CD at the DHEA concentration of 10 g/L. The study also showed that the mechanisms of CDs complexation technique for enhancing the hydroxylation yield from DHEA by *C. lini* ST-1 included not only solubilizing of steroids, but also increasing cell permeability for both substrate and product. To the best of our knowledge, this study was the first report on microbial transformation of DHEA by *C. lini* ST-1 with CDs.

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