J Incl Phenom Macrocycl Chem (2011) 69:273–280
DOI 10.1007/s10847-010-9844-y

ORIGINAL ARTICLE

Characterization of the inclusion complex of 16,17α-epoxyprogesterone with randomly methylated β-cyclodextrin in aqueous solution and in the solid state

Bing Ma · Yanbing Shen · Zhi Fan · Yu Zheng · Hua Sun · Jianmei Luo · Min Wang

Received: 9 July 2010 / Accepted: 11 August 2010 / Published online: 26 August 2010

Abstract The inclusion complex between 16,17α-epoxyprogesterone (EP) and randomly methylated β-cyclodextrin (RM-β-CD) was prepared by freeze-drying method and subsequently characterized by means of differential scanning calorimetry, X-ray powder diffractometry, scanning electron microscopy and Fourier transform infrared spectroscopy techniques. The results clearly revealed that EP molecules were included in the cavity of RM-β-CD, thus giving rise to the solubility enhancement of EP in aqueous solution. The effect of RM-β-CD on the aqueous solution of EP was evaluated by the phase solubility diagram. The amount of EP increased linearly with the addition of RM-β-CD according to an A_L type plot, indicating the formation of 1:1 stoichiometric inclusion complexes. Thermodynamic study showed the complexation process between EP and RM-β-CD ($\Delta G = -34.4$ kJ mol^{-1}) was driven by both favorable enthalpy ($\Delta H = -21.4$ kJ mol^{-1}) and entropy ($\Delta S = 43.2$ J mol^{-1} K^{-1}) changes. Dissolution studies showed that such inclusion complex offered a marked improvement in the dissolution rate compared to EP alone and the physical mixture.

B. Ma · Y. Shen · Y. Zheng · H. Sun · J. Luo · M. Wang (✉)
Key Laboratory of Industrial Microbiology, Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, No. 29, 13th. Avenue TEDA, Tianjin 300457, People's Republic of China
e-mail: minw@tust.edu.cn

Z. Fan
College of Sciences, Tianjin University of Science and Technology, No. 29, 13th. Avenue, TEDA, Tianjin 300457, People's Republic of China

Keywords 16,17α-Epoxyprogesterone · Randomly methylated β-cyclodextrin · Inclusion complex · Phase solubility diagram · Thermodynamic · Dissolution

Introduction

Steroids are a class of compounds that play an important part in many biological processes and a variety of steroid drugs are widely used as anti-inflammatory, diuretic, contraceptive, progestational, anti-androgenic, and anticancer agents [1]. Microbial reaction for the steroid transformation is a typical example of the successful application of microbial technology in large-scale industrial process and some specific microbial transformation steps have replaced the chemical reactions in the production of therapeutically useful drugs and hormones in recent years.

16,17α-Epoxyprogesterone (EP) is an important steroid (Fig. 1) that serves as an intermediate for many hormone pharmaceuticals, such as hydrocortisone and megestrol, through 11α-hydroxylation by *Rhizopus nigricans* in industry. Many reports on this bioconversion had been issued [2, 3]. However, the solubility of the substrate EP with a remarkable hydrophobic nature is very low, which is considered as the rate-limiting step of steroid biotransformation [4]. An alternative way to improve substrate solubility is adding organic solvent into media. Nevertheless, for whole-cell system, the organic solvent might lead to the disruption of the cell membrane, denaturation of membrane bound enzymes, even to cytolysis [5]. Therefore, the key issue on the steroids biotransformation is to find a kind of medium to not only improve the solubility and dissolution rate of the substrate but also exhibit a good biocompatibility.

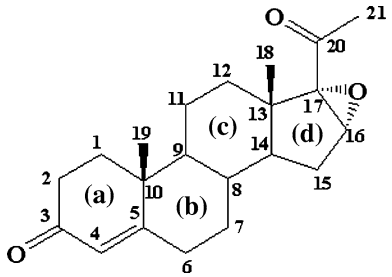

Fig. 1 Chemical structure of 16,17α-epoxyprogesterone. The carbon atoms of the steroid scaffold are numbered, and the four rings are labeled (*A*), (*B*), (*C*), and (*D*)

Cyclodextrins (CDs) have been widely used to form inclusion complexes with a variety of guest compounds through non-covalent interactions to enhance their water solubility, stability, physicochemical incompatibilities, oral absorption or bioavailability for the hydrophobic nature of the interior cavity [6–10]. Additionally, CDs are inert to microorganisms and have a benefit to respiratory-chain activity [11]. Owing to these unique properties, CDs have been intensively added in liquid cultures to facilitate and enhance microbial transformations of steroids through the inclusion complex formation between CDs and the substrate, as shown in previous reports [12–15]. Randomly methylated β-cyclodextrin (RM-β-CD) is a kind of common and typical amorphous β-CD derivate. Methylation on the hydroxyl units breaking some of the intramolecular hydrogen bonds favorably extends the hydrophobic cavity without any steric hindrance and provides greater inclusion ability [16]. Moreover, the solubility of RM-β-CD at room temperature (>2,000 mg/mL) is significantly higher than that of β-CD (18.5 mg/mL) in aqueous solution [17]. It is reported that the specific properties of RM-β-CD in aqueous solution could contribute to a higher solubility and dissolution of the hydrophobic compounds when in the complexed state [18, 19].

However, the comprehensive understanding of the effect of CDs on steroid bioconversion through the formation of inclusion complex is rather limited up to now. Few of the mechanism of host–guest interactions in the CD-promoted biotransformation had been investigated.

In the present study, the inclusion complex of EP and RM-β-CD was prepared and characterized by differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). The extent and mode of solubility enhancement exerted by RM-β-CD in solution were experimentally measured by phase solubility diagram. The effects of temperature on the interaction were examined and the thermodynamic parameters were estimated to evaluate the driving forces for complex formation. Furthermore, the dissolution profiles of EP, the EP/RM-β-CD physical mixture and their inclusion complex were measured and compared. The results should be significant in the study of CD-promoted biotransformation of steroids, as well as in the application of CDs in drug delivery.

Materials and methods

Materials

EP was supplied by Tianjin Pharmaceutical Company. Standard EP was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). 2-hydroxypropyl-β-cyclodextrin (HP-β-CD, M.W. = 1,400, D.S. = 0.58–0.73) and RM-β-CD (M.W. = 1,310 and D.S. = 1.6–1.9) were obtained from Wacker Fine Chemical Corporation (Germany). All other reagents were of BDH or HPLC reagent grade and water used throughout the study was deionized and double-distilled.

Preparation of inclusion complexes and physical mixtures

Equimolar EP and RM-β-CD were employed for the preparation of the solid inclusion complexes and physical mixtures. The inclusion complexes were prepared by the freeze-drying method. The required quantity of EP was dissolved in little ethanol and added to an aqueous solution of RM-β-CD. The mixed solution was stirred for 2 days with a magnetic stirrer at room temperature. Then the resulting solution was frozen at −70 °C and lyophilized in a freeze-dryer. The physical mixture for the purpose of comparison was also prepared by simply admixing the powders of both EP and RM-β-CD in a mortar with pestling for 5 min.

Differential scanning calorimetry

The thermal behaviors of all samples of RM-β-CD, EP, their physical mixtures and EP/RM-β-CD complex were examined using differential scanning calorimetry (DSC) (Seiko 2400, Japan). An accurately weighed sample of each solid, equivalent to 5 mg EP, was heated in a sealed aluminum pan, using an empty pan sealed as reference, over the temperature range from 30 to 300 °C at a rate of 10 °C/min. An indium standard was used for temperature calibration.

X-ray powder diffractometry

The X-ray powder diffractometry (XRPD) patterns were measured with an X-ray diffractometer (Philips PW1729,

Japan). Radiation generated from a Cu Kα source and filtered through Ni filters with a wavelength of 1.79025 Å at 40 mA and 30 kV was used. The instrument was operated with a scanning rate of 0.02°/s^{-1} over the 2θ range of 3–50°. The powder diffraction patterns of RM-β-CD, EP, physical mixture and inclusion complexes were recorded.

Scanning electron microscopy

The surface morphology of RM-β-CD, EP, physical mixture and inclusion complex were visualized using Scanning Electron Microscope (SEM) (JSM-6380, JEOL, USA). The samples were mounted on a brass stub using double sided tape and then sputtered with a thin layer of gold. The photographs were taken at an acceleration voltage of 20 kV.

Fourier transform infrared spectroscopy

Thin pellets containing 1 mg of each sample dispersed in 100 mg of KBr were used for Fourier transform infrared spectroscopy (FTIR) measurements. The spectra were recorded at room temperature as an average of 32 scans by PerkinElmer Model 1600 FT-IR spectrometer (Wellesley, USA) in the range of 4,000–400 cm^{-1} at 4 cm^{-1} resolution.

High performance liquid chromatography

The sample was analyzed by an Agilent 1200 high performance liquid chromatography (HPLC) system (USA) to evaluate the concentration of EP dissolved with measuring absorbance at 240 nm. Samples were eluted with a mixture of methanol and water (9:1 volume/volume) on Comatex C_{18} reversed-phase column (250 mm × 4.6 mm, 5 µm particle size) using a 1.0 mL/min flow rate. The column temperature was maintained at 28 °C and the sample volumes injected were 20 µL. The concentrations of EP were determined from calibration curves, making from eluent solutions of standard EP.

Phase solubility studies

Phase solubility studies were carried out in water at 20, 25, 28, 35 and 40 °C, respectively, according to the method reported by Higuchi and Connors [20]. An excess of EP was added to 10 mL of aqueous solution with various concentrations of CDs, ranging from 0 to 100 mM for HP-β-CD and RM-β-CD. The resulting suspensions were shaken for 48 h. After equilibration, the suspensions were filtered through 0.45 µm membrane filters, appropriately diluted with the mobile phase and the total concentration of the EP in the filtrate was analyzed by HPLC. The apparent stability constants ($K_{1:1}$) were calculated from the phase solubility diagram according to the following equation:

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where slope is the value found in the linear regression and S_0 is the solubility of EP in the absence of CDs.

Estimation of thermodynamic parameters

Gibbs and Van't Hoff equations were used to estimate the thermodynamic parameters ΔH, ΔS, and ΔG according to:

$$\Delta G = \Delta H - T\Delta S \quad (2)$$

$$\ln(K) = \Delta S/R - \Delta H/RT \quad (3)$$

A plot of $\ln(K)$ versus $1/T$ produces: slope $= -\Delta H/R$ and intercept $= \Delta S/R$.

K represents either $K^x_{1:1}$ or S^x_0, and x stands for the mole fraction standard state. $K_{1:1}$ (M^{-1}) and S_0 (M) were transformed to the dimensionless mole fraction units $K^x_{1:1}$ and S^x_0 by multiplying $K_{1:1}$ with 55.5 and dividing S_o by 55.5, where 55.5 represents the number of moles of water in 1 L water [21, 22].

Dissolution studies

The dissolution studies were performed using a ZRS-8G dissolution tester (Tianda Tianfa Technology Co. Ltd., Tianjin, China) according to the USP XXIII method (apparatus II-paddle) at a temperature of 28 °C and a stirring rate of 100 rpm. Accurately weighed samples (EP 100 mg or its equivalent amount of physical mixture or complex) were added to 1000 mL of water. Aliquots of 5 mL samples were withdrawn at different time intervals. The solutions were immediately filtered through 0.45 µm membrane filters, suitably diluted, and the concentrations of EP were determined by HPLC. In the meantime, an equal volume of water of the same temperature was added to keep constant volume. Each experiment was carried out in triplicate.

Results and discussion

Differential scanning calorimetry

DSC is a fast and relatively inexpensive technique to characterize cyclodextrin complexes. Figure 2 illustrates the DSC thermograms of RM-β-CD, EP, their physical mixtures and EP/RM-β-CD inclusion complex. The DSC curve of RM-β-CD showed a shallow and broad endothermic peak in the range of 75–154 °C, which could be

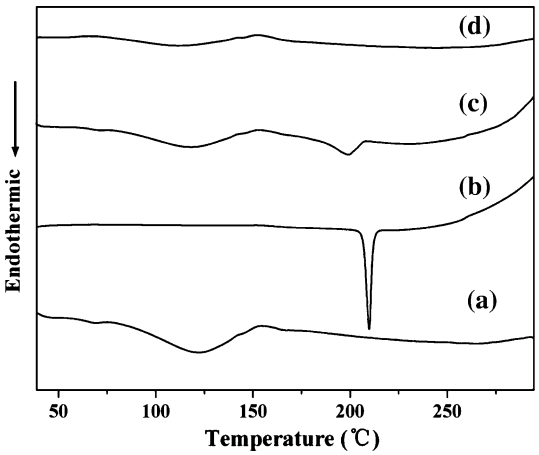

Fig. 2 Differential scanning calorimetry curves of (*a*) RM-*β*-CD, (*b*) EP, (*c*) physical mixture of EP and RM-*β*-CD, and (*d*) EP/RM-*β*-CD inclusion complex

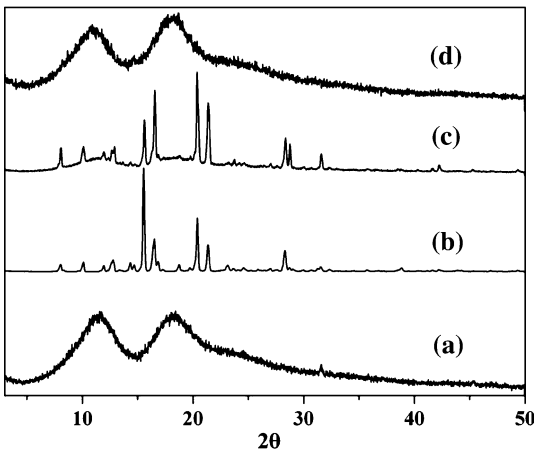

Fig. 3 XRPD patterns of (*a*) RM-*β*-CD, (*b*) EP, (*c*) physical mixture of EP and RM-*β*-CD, and (*d*) EP/RM-*β*-CD inclusion complex

attributed to the release of water molecule from the cavity (desolvation). The thermogram of EP was typical of a highly crystalline compound, characterized by a sharp endothermic peak at 209 °C corresponding to its melting point. In the physical mixture system, the DSC pattern revealed the presence of peaks of both pure compounds except that the melting endotherm of EP slightly shifted to 201 °C with a decrease in the peak intensity. However, in case of the inclusion complex sample, a large diminution of the dehydration effect of RM-*β*-CD was observed and the characteristic melting peak of crystalline EP was lost, indicating that the crystalline steroid was converted to amorphous state and dispersed at a molecular level inside the cyclodextrin cavity.

X-ray powder diffractometry

XRPD is a useful method for the detection of cyclodextrin complexation in powder or microcrystalline states. The XRPD patterns of RM-*β*-CD, EP, their physical mixtures and EP/RM-*β*-CD inclusion complex are shown in Fig. 3. No diffraction peak was apparent with RM-*β*-CD, suggesting that it is essentially amorphous. However, the diffractogram of EP exhibited a series of intense and sharp peaks, indicative of its crystalline nature. The XRPD pattern of the physical mixture confirmed the presence of both species as isolated solids, as the diffractogram exhibited both EP peaks and the amorphous halo of RM-*β*-CD. The diffraction pattern of the complex should be clearly distinct from that of the superimposition of each of the components if a true inclusion complex has been formed [23]. The XRPD pattern of the EP/RM-*β*-CD inclusion complex showed a completely diffused pattern with no diffraction peaks of EP. These differences represented a loss of crystallinity with the formation of a less organized system, indicating the formation of a real amorphous inclusion complex between EP and RM-*β*-CD. Similar results were found by Hirlekar and Kadam [16], Ribeiro et al. [18] and Yang et al. [24].

Scanning electron microscopy

The morphology of RM-*β*-CD, EP, physical mixture and inclusion complex is shown in Fig. 4. RM-*β*-CD was composed by spherical particles with smooth surface, an observation similar to that made by Ribeiro et al. [18] and Pravin et al. [19], whereas EP presented regular rectangle-shaped crystal. The SEM image of the physical mixture system showed clearly the characteristic EP crystals which were mixed with the cyclodextrin particles, thus confirming the presence of crystalline steroid. However, in the freeze-dried EP/RM-*β*-CD system, the original morphology of the parent compounds disappeared and it was impossible to differentiate between the two components. The freeze-dried products appeared to have less crystalline structure with a uniform appearance. This drastic change in particle shape and aspect in the freeze-dried product is indicative of the presence of a new solid phase. Although the SEM technique is inadequate to conclude genuine complex formation, the obtained images support the consecution of a new single component [25].

Fourier transform infrared spectroscopy

Figure 5 shows the Infrared spectra of RM-*β*-CD, EP, physical mixture and the inclusion complex. The IR spectrum of EP is characterized in particular by three sharp signals at 1611, 1661, 1699 cm^{-1}. The peaks at 1661, 1699 cm^{-1} are assigned to carbonyl-stretching vibration bands of C_3 and C_{20} in EP, respectively [26, 27]. The

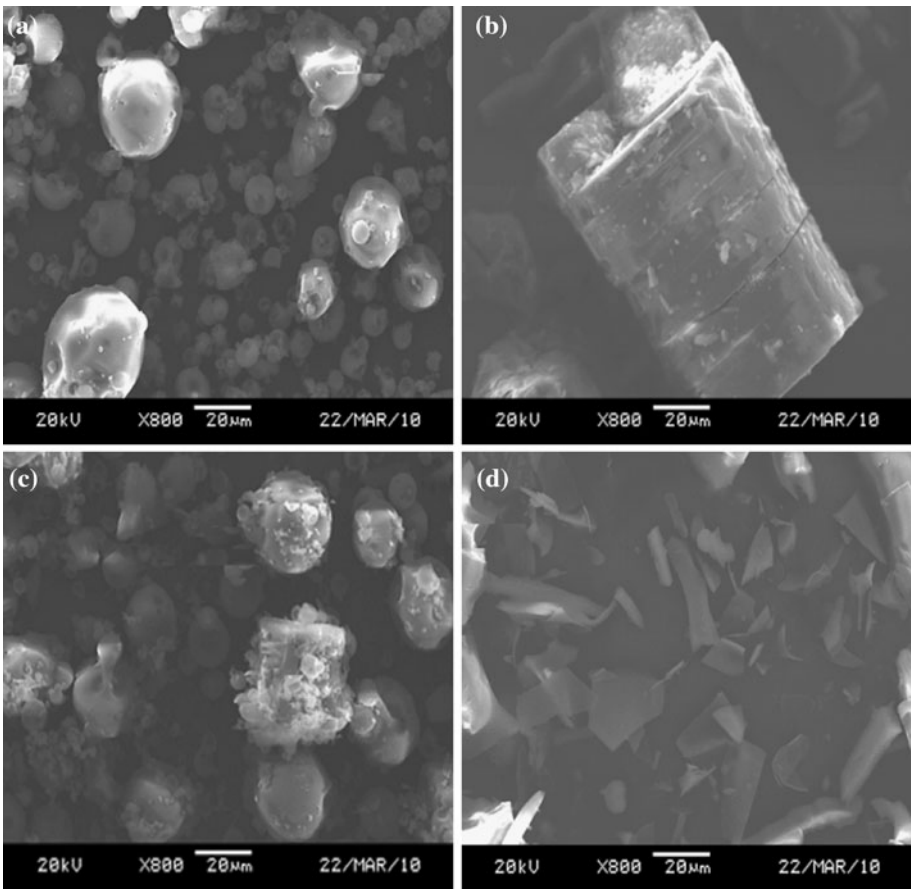

Fig. 4 Scanning electron microphotographs of **a** RM-β-CD, **b** EP, **c** physical mixture of EP and RM-β-CD, and **d** EP/RM-β-CD inclusion complex

absorption band observed at 1611 cm^{-1} corresponds to the conjugated vinylic group (C=C) in the A ring of EP.

The spectrum of the physical mixture exhibited spectrum corresponding to a superposition of their parent components. By comparison of the IR spectrograms of EP and RM-β-CD, we can see that the spectrum of the physical mixture showed no significant differences from the respective spectra of each of the pure components. Whereas in the IR spectra of inclusion complex, there was a large change in the characteristic bands absorption of EP. The band of methyl-keto group (C_{20}=O) reduced its intensity dramatically and shifted to 1704 cm^{-1} suggesting a strong interaction between this side aliphatic chain of EP and the RM-β-CD ring. The cyclic carbonyl (C_3=O) stretching bands and the vinylic group band disappeared completely. The band located at 1635 cm^{-1} in the IR spectra of RM-β-CD which were correlated to δ-HOH bending of water molecules attached to cyclodextrins shifted to 1641 cm^{-1} in that of the inclusion complex. On the basis of these results, it can be concluded preliminarily that the A-ring of the steroid was probably enclosed in the RM-β-CD cavity.

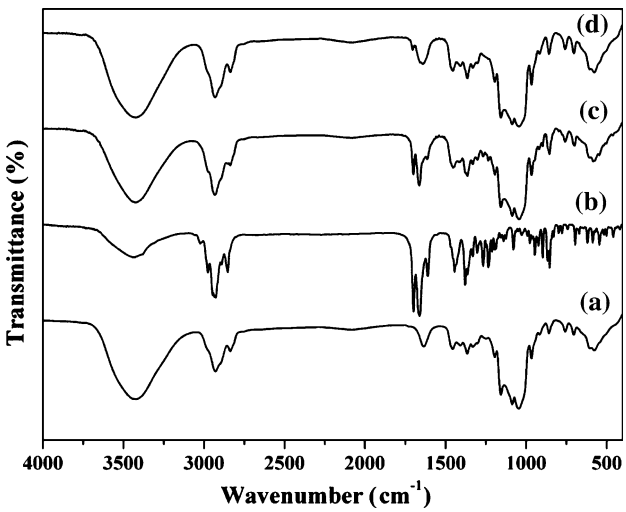

Fig. 5 FTIR spectra of (*a*) RM-β-CD, (*b*) EP, (*c*) physical mixture of EP and RM-β-CD, and (*d*) EP/RM-β-CD inclusion complex

Therefore, FTIR results, along with those of DSC, XRPD and SEM, confirm the presence of inclusion complex in the solid state.

Phase solubility studies

Phase solubility diagrams of EP with HP-β-CD and RM-β-CD obtained in water at 28 °C are shown in Fig. 6. Both RM-β-CD and HP-β-CD were found to enhance water

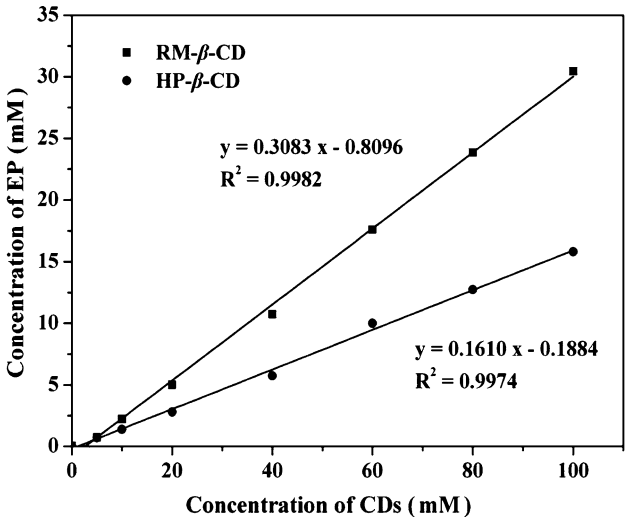

Fig. 6 Phase solubility diagrams of EP with CDs in aqueous solution at 28 °C

solubility of EP linearly with increasing CDs concentrations (0–100 mM) and showed typical A_L type diagrams according to Higuchi and Connors [20]. The slope values (Table 1) were lower than one indicating that water soluble inclusion complexes with 1:1 stoichiometry between the guest and the host were formed in the range of CDs concentration studied.

As shown in Table 1, the values of the apparent stability constant $K_{1:1}$ and the enhancement factor (EF) followed the order of: RM-β-CD > HP-β-CD. The results suggested that the interaction between EP and CDs related to the spatial relationship between the host and guest molecules (steric and hydrophobic factors). Relatively higher values of $K_{1:1}$ and EF for RM-β-CD, compared with HP-β-CD, indicated that EP formed more stable complexes with the former which might be attributed to the extension of the hydrophobic cavity without steric hindrance and provision of greater inclusion ability [19]. On the other hand, the smaller ones for HP-β-CD represented the lower binding potential. It was the contribution of the presence of hydroxypropyl substituent which partially covered the opening of cavity and thus hampered the inclusion of EP into it [28].

Table 1 Complex formation parameters for EP/CDs systems in water at 28 °C

CD	Type of diagram	Slope	$K_{1:1}$ (M^{-1})	EF
RM-β-CD	A_L	0.3083	17142	85.1
HP-β-CD	A_L	0.1610	7380	53.2

The enhancement factor (EF) = S_{eq}/S_0, where S_{eq} and S_0 are the solubilities of EP in the presence (10 mM) and absence of CDs, respectively

Table 2 The inherent EP solubility (S_0) and the complex formation stability constants ($K_{1:1}$) obtained in water at different temperatures

t (°C)	S_0 (mM)	$K_{1:1}$ (M^{-1})
20.0	0.015	21110
25.0	0.021	18949
28.0	0.026	17142
35.0	0.043	13379
40.0	0.060	12481

Thermodynamics

The effects of temperature on the solubilizing capacity of RM-β-CD in water were studied and the relevant thermodynamic parameters of such interactions were calculated.

The complex formation stability constants ($K_{1:1}$) and the inherent solubility S_0 in the absence of RM-β-CD are listed in Table 2. The phase solubility diagrams of EP/RM-β-CD obtained in water at different temperatures are shown in Fig. 7a. Although the solubility of EP increased upon increasing the temperature from 20.0 to 40.0 °C, the stability constant values decreased. This was not unexpected since cyclodextrin complexes usually dissociate upon increasing the temperature of the solution. Van't Hoff plots of $\ln K^x_{1:1}$ and $\ln S^x_0$ (where x denotes the mole fraction standard state) against $1/T$ were constructed and are shown in Fig. 7b, while the relevant thermodynamic parameters (ΔH, ΔS, and ΔG) were obtained are listed in Table 3. Such treatment provided further illustration of the effects of temperature on the stability constants of EP/RM-β-CD complexes. The linearity of the Van't Hoff profiles proved that the enthalpy change (ΔH) and entropy change (ΔS) are constant over the temperature range investigated.

The solubility of EP ($\Delta G = 36.4$ kJ mol^{-1}) is impeded by enthalpy ($\Delta H = 53.1$ kJ mol^{-1}) and favored by entropy changes ($\Delta S = 55.4$ J mol^{-1} K^{-1}). The positive enthalpy indicates that the dissolving process is endothermic, where S_0 increases with increasing temperature. On the other hand, the positive entropy changes result in a decrease in the order of EP in solution [21, 22]. The negative value of the Gibbs energy at 301 K ($\Delta G = -34.4$ kJ mol^{-1}) suggests that the inclusion process is a spontaneous one. Complex formation for EP and RM-β-CD is driven by both favorable enthalpy ($\Delta H = -21.4$ kJ mol^{-1}) and entropy ($\Delta S = 43.2$ J mol^{-1} K^{-1}) changes. The negative value of the enthalpy changes implies that the interaction process of EP with RM-β-CD is exothermic. An exothermic enthalpy is attributed to van der Waals interactions, hydrogen binding between the hydroxyl groups of cyclodextrins and the "guest", or due to the release of "high-enthalpy water molecules" from the inner CD cavity into the solution [29], whereas positive entropy indicates that the hydrophobic

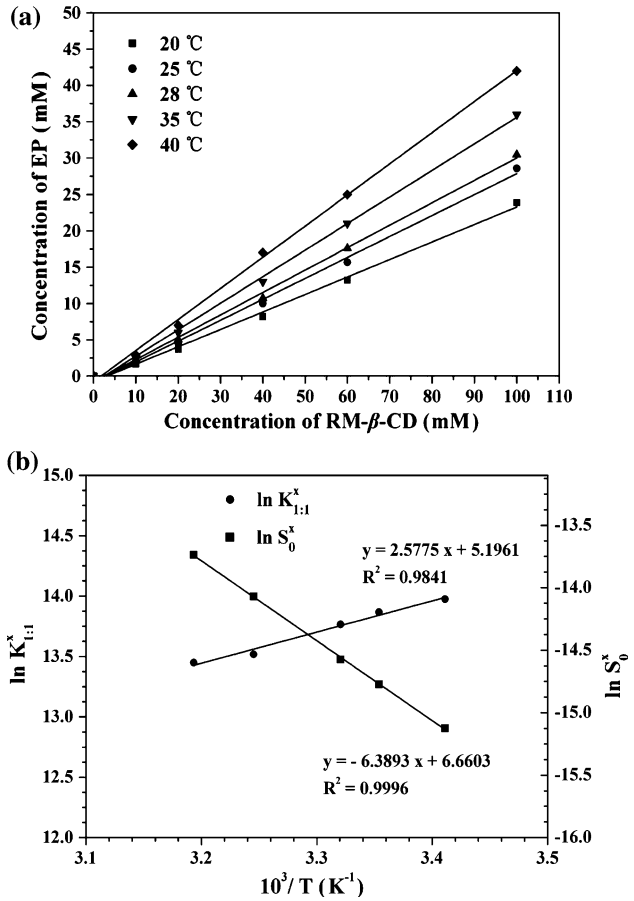

Fig. 7 **a** Phase solubility diagrams of EP/RM-β-CD obtained in water at different temperatures, and **b** the corresponding Van't Hoff plots of $\ln K^{x}_{1:1}$ and $\ln S^{x}_{0}$ against $1/T$ (x stands for the mole fraction standard state)

Table 3 Thermodynamic parameters of EP/RM-β-CD complex obtained in water at 28 °C

	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
Solubility of EP (S_0)	36.4	53.1	55.4
EP/RM-β-CD complex	−34.4	−21.4	43.2

effect (desolvation) is a strong driving force for EP/RM-β-CD inclusion complexation [21].

Dissolution studies

The dissolution profiles of EP and its binary systems are reported in Fig. 8. The improvement in the dissolution was as follows: EP < physical mixture < complex. Dissolution of EP did not reach saturation equilibrium even after 240 min. Physical mixture yielded a dissolution profile that was higher than that of EP. This might be attributed to improvement of the wettability and solubility of EP

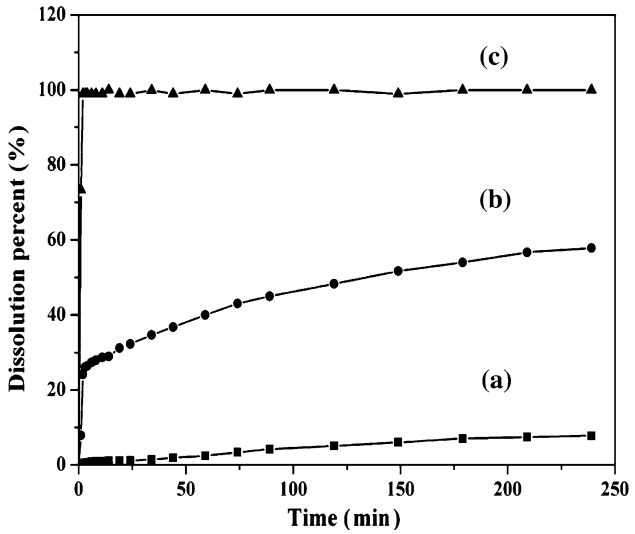

Fig. 8 Dissolution profiles of (*a*) EP, (*b*) physical mixture, and (*c*) inclusion complex in water at 28 °C (n = 3)

resulting from the coexistence of RM-β-CD in the dissolution medium. The dissolution curve of complex exhibited a much faster initial rate and higher solubility as compared to free EP and physical mixture. Only about 0.4% of EP could be dissolved from the steroid alone and 24% from the physical mixture within 2 min. However, at the same time interval, EP was rapidly released from the complex and the dissolution percent reached 99.2%. A very high increase of EP dissolution rate from inclusion complex may be probably due to partial entrapment of EP molecule inside the RM-β-CD torus which imparts its hydrophilic character and hence, increases its solubility and wettability. Additionally, it seemed that changing of the steroid from crystalline agglomerates to amorphous state, as seen from DSC and XRPD, is another factor that led to dissolution enhancement in the complex system.

Conclusion

In summary, the study of the inclusion of EP into RM-β-CD indicated the formation of a 1:1 complex. It has been found that the water solubility of EP was increased by inclusion with RM-β-CD according to the phase solubility diagram. Complex formation of EP with RM-β-CD was driven by both favorable enthalpy and entropy changes. Dissolution studies showed that such inclusion complex offered a significant improvement in the dissolution rate compared to EP alone and the physical mixture. Taking into consideration that the solubility of the substrate EP is very low, which may limit the maximal usefulness of the steroid microbial transformation, the strategy could be successfully employed in the design of novel formulation of EP in microbial transformation.

Acknowledgements This work is financially supported by Nature Science Foundation of China (NSFC, Grant No. 20776111), and Program for New Century Excellent Talents in University (NCET-08-0911), which are gratefully acknowledged.

References

1. Mahato, S.B., Garai, S.: Advances in microbial steroid biotransformation. Steroids **62**, 332–345 (1997)
2. Breskvar, K., Ferenčak, Z., Hudnik-Plevnik, T.: The role of cytochrome $P450_{11\alpha}$ in detoxification of steroids in the filamentous fungus *Rhizopus nigricans*. J. Steroid Biochem. Mol. Biol. **52**, 271–275 (1995)
3. Zhou, H., Lu, W., Wen, J., Ma, L.: Kinetic analysis of 11α-hydroxylation of steroids by *Rhizopus nigricans*. J. Mol. Catal. B: Enzym. **56**, 136–141 (2009)
4. Sedlaczek, L., Jaworski, A., Wilmańska, D.: Transformation of steroids by fungal spores. Appl. Microbiol. Biotechnol. **13**, 155–160 (1981)
5. Cruz, A., Fernandes, P., Cabral, J.M.S., Pinheiro, H.M.: Whole-cell bioconversion of β-sitosterol in aqueous-organie two-phase systems. J. Mol. Catal. B: Enzym. **11**, 579–585 (2001)
6. Al-Omar, A., Abdou, S., De Robertis, L., Marsura, A., Finance, C.: Complexation study and anticellular activity enhancement by doxorubicin-cyclodextrin complexes on a multidrug-resistant adenocarcinoma cell line. Bioorg. Med. Chem. Lett. **9**, 1115–1120 (1999)
7. Shehatta, I.: Cyclodextrins as enhancers of the aqueous solubility of the anthelmintic drug mebendazole: thermodynamic considerations. Monatsh. Chem. **133**, 1239–1247 (2002)
8. Szejtli, J., Szente, L.: Elimination of bitter, disgusting tastes of drugs and foods by cyclodextrins. Eur. J. Pharm. Biopharm. **61**, 115–125 (2005)
9. Yang, B., Yang, L.J., Lin, J., Chen, Y., Liu, Y.: Binding behaviors of scutellarin with α-, β-, γ-cyclodextrins and their derivatives. J. Incl. Phenom. Macrocycl. Chem. **64**, 149–155 (2009)
10. Uzqueda, M., Martín, C., Zornoza, A., Sánchez, M., Vélaz, I.: Physicochemical characterization of terbinafine-cyclodextrin complexes in solution and in the solid state. J. Incl. Phenom. Macrocycl. Chem. **66**, 393–402 (2010)
11. Fokina, V.V., Karpov, A.V., Sidorov, I.A., Andrjushina, V.A., Arinbasarova, A.Y.: The influence of β-cyclodextrin on the kinetics of 1-en-dehydrogenation of 6α-methylhydrocortisone by *Arthrobacter globiformis* cells. Appl. Microbiol. Biotechnol. **47**, 645–649 (1997)
12. Roglič, U., Žnidaršič-Plazl, P., Plazl, I.: The influence of β-cyclodextrin on the kinetics of progesterone transformation by *Rhizopus nigricans*. Biocatal. Biotransform. **23**, 299–305 (2005)
13. Roglič, U., Plazl, I., Žnidaršič-Plazl, P.: Batch and continuous transformation of progesterone by *Rhizopus nigricans* pellets in the presence of β-cyclodextrin. Biocatal. Biotransform. **25**, 16–23 (2007)
14. Manosroi, J., Saowakhon, S., Manosroi, A.: Enhancement of 17α-hydroxyprogesterone production from progesterone by biotransformation using hydroxypropyl-β-cyclodextrin complexation technique. J. Steroid Biochem. Mol. Biol. **112**, 201–204 (2008)
15. Zhang, L.T., Wang, M., Shen, Y.B., Ma, Y.H., Luo, J.M.: Improvement of steroid biotransformation with hydroxypropyl-β-cyclodextrin induced complexation. Appl. Biochem. Biotechnol. **159**, 642–654 (2009)
16. Hirlekar, R., Kadam, V.: Preparation and characterization of inclusion complexes of carvedilol with methyl-β-cyclodextrin. J. Incl. Phenom. Macrocycl. Chem. **63**, 219–224 (2009)
17. Hedges, A.R.: Industrial applications of cyclodextrins. Chem. Rev. **98**, 2035–2044 (1998)
18. Ribeiro, A., Figueiras, A., Santos, D., Veiga, F.: Preparation and solid-state characterization of inclusion complexes formed between miconazole and methyl-β-cyclodextrin. AAPS PharmSciTech. **9**, 1102–1109 (2008)
19. Pravin, N., Babasaheb, A., Neha, D., Vilasrao, K., Rajashree, H.: Solid state characterization of the inclusion complex of valsartan with methyl β-cyclodextrin. J. Incl. Phenom. Macrocycl. Chem. **65**, 377–383 (2009)
20. Higuchi, T., Connors, K.A.: Phase-solubility techniques. Adv. Anal. Chem. Instr. **4**, 117–212 (1965)
21. Al Omari, M.M., Zughul, M.B., Davies, J.E.D., Badwan, A.A.: Effect of buffer species on the inclusion complexation of acidic drug celecoxib with cyclodextrin in solution. J. Incl. Phenom. Macrocycl. Chem. **55**, 247–254 (2006)
22. Al Omari, M.M., Daraghmeh, N.H., El-Barghouthi, M.I., Zughul, M.B., Chowdhry, B.Z., Leharne, S.A., Badwan, A.A.: Novel inclusion complex of ibuprofen tromethamine with cyclodextrins: Physico-chemical characterization. J. Pharm. Biomed. Anal. **50**, 449–458 (2009)
23. Naidu, N.B., Chowdary, K.P.R., Murthy, K.V.R., Satyanarayana, V., Hayman, A.R., Becket, G.: Physicochemical characterization and dissolution properties of meloxicam-cyclodextrin binary systems. J. Pharm. Biomed. Anal. **35**, 75–86 (2004)
24. Yang, B., Lin, J., Chen, Y., Liu, Y.: Artemether/hydroxypropyl-β-cyclodextrin host–guest system: characterization, phase-solubility and inclusion mode. Bioorgan. Med. Chem. **17**, 6311–6317 (2009)
25. Sathigari, S., Chadha, G., Lee, Y.H., Wright, N., Parsons, D.L., Rangari, V.K., Fasina, O., Babu, R.J.: Physicochemical characterization of efavirenz-cyclodextrin inclusion complexes. AAPS PharmSciTech. **10**, 81–87 (2009)
26. Uekama, K., Fujinaga, T., Hirayama, F., Otagiri, M., Yamasaki, M.: Inclusion complexations of steroid hormones with cyclodextrins in water and in solid phase. Int. J. Pharm. **10**, 1–15 (1982)
27. Cerchiara, T., Luppi, B., Bigucci, F., Zecchi, V.: Effect of chitosan on progesterone release from hydroxypropyl-β-cyclodextrin complexes. Int. J. Pharm. **258**, 209–215 (2003)
28. Mura, P., Zerrouk, N., Faucci, M.T., Maestrelli, F., Chemtob, C.: Comparative study of ibuproxam complexation with amorphous β-cyclodextrin derivatives in solution and in the solid state. Eur. J. Pharm. Biopharm. **54**, 181–191 (2002)
29. Ghuzlaan, A., Al Omari, M.M., Al-Sou'od, K.A.: Prednisone/cyclodextrin inclusion complexation: phase solubility, thermodynamic, physicochemical and computational analysis. J. Solut. Chem. **38**, 83–94 (2009)