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Short communication

Fed-batch production of (*S*)-flurbiprofen in lipase-catalyzed dispersed aqueous phase reaction system induced by succinyl β-cyclodextrin and its extractive purification

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Abstract

Optically active (*S*)-flurbiprofen was produced fed-batch-wisely in a lipase-catalyzed dispersed aqueous phase reaction system induced by succinyl β-cyclodextrin (suβ-CD). A highly concentrated 480 mM (*S*)-flurbiprofen, corresponding to 117.0 g/l, with an enantiomeric excess of 0.98 and conversion yield of 0.48 was obtained. (*S*)-Flurbiprofen produced in an inclusion complex form with suβ-CD was extractively purified using three-step procedures: decomplexation of (*S*)-flurbiprofen and residual (*R*)-flurbiprofen ethyl ester ((*R*)-FEE) using the ethyl acetate, dissolution of (*S*)-flurbiprofen from (*R*)-FEE using a sodium bicarbonate solution, and selective precipitation of (*S*)-flurbiprofen using 2-propanol. Consequently, an extremely high concentration of 420 mM (*S*)-flurbiprofen with an optical purity higher than 98% was recovered after purification.

Keywords: (*S*)-Flurbiprofen; Fed-batch; (*R,S*)-Flurbiprofen ethyl ester; Lipase; Succinyl β-cyclodextrin; Inclusion complex; Extractive purification

2-Arylpropionic acid (profen), a non-steroidal anti-inflammatory drug, exhibits the pharmacological activity when it exists as an optically active (*S*)-enantiomer, however, it has been commercially produced as a racemic mixture by chemical synthesis [1,2]. However, the kinetic resolution of enantiopure (*S*)-profen from a racemic profen mixture using lipase or esterase as the chiral biocatalyst has drawn considerable attention recently [3,4].

2-Arypropionic acid derivatives are usually water immiscible compounds. Thus, to increase the enantioselectivity of biocatalysts and overcome the low solubility of immiscible substrate *rac*-2-arypropionic acid derivatives during the enzymatic resolutional reaction, various methods have been applied, including the modification of the lipase using organic solvents [5,6] and the utilization of a surfactant [7], cyclodextrins [8], and crown ether [9].

In our previous works [10,11], the chiral cyclodextrin derivative hydroxypropyl-β-cyclodextrin (HP-β-CD) was applied as the dispenser and chiral selector for the kinetic resolution of (*R,S*)-ketoprofen ethyl ester using a lipase from *Candida rugosa*. As a result, a very high concentration of optically pure (*S*)-ketoprofen, as high as 243 mM, was produced, and the enantiomeric excess and conversion yield also exceeded 0.99 and 0.49, respectively.

Also, the chiral succinyl β-cyclodextrin (suβ-CD) was applied for the kinetic resolution of (*R,S*)-flurbiprofen ethyl ester ((*R,S*)-FEE) to (*S*)-flurbiprofen. An extremely high 240 mM of (*S*)-flurbiprofen was obtained in above dispersed aqueous phase reaction system induced by suβ-CD, with an enantiomeric excess of 0.98 and conversion yield of 0.48. The resolution characteristics were investigated by comparing the inclusion complex formability of each (*S*)- and (*R*)-forms of flurbiprofen ethyl ester with suβ-CD by a phase-solubility diagram, DSC, and ^{1}H NMR [12].

However, the optically pure (*S*)-flurbiprofen has been produced as an inclusion complex form entrapped in the cavity of the suβ-CD molecule used as the dispenser and chiral selector,

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the entrapped (*S*)-flurbiprofen needs to be extractively purified. Also, (*S*)-flurbiprofen has to be produced even higher concentration in order to meet the cost effectiveness for the development of an industrially feasible process.

In this work, optically pure (*S*)-flurbiprofen was enantioselectively produced by feeding the immiscible (*R,S*)-FEE fed-batch-wisely up to an extremely high concentration, and it was purified by three-step procedures: decomplexation of (*S*)-flurbiprofen and residual (*R*)-FEE from suβ-CD, dissolution of (*S*)-flurbiprofen from (*R*)-FEE, and selective precipitation of (*S*)-flurbiprofen.

The biocatalyst used for biosynthesis of the optically active (*S*)-flurbiprofen was the lipase from *C. rugosa* (Sigma Co., St. Louis, USA). The (*R,S*)-FEE was synthesized from (*R,S*)-flurbiprofen (Kolon Co., Seoul, Korea) using an esterification reaction [12,13], while the suβ-CD used as the dispenser and chiral selector was purchased from Cyclolab (Cyclolab Ltd., Budapest, Hungary).

The initial batch reaction was carried out using 500 mM (*R,S*)-FEE, 600 units of *C. rugosa* lipase per 1 mmol of (*R,S*)-FEE, and 1000 mM suβ-CD in a 50 mM sodium phosphate buffer (pH 7.0) at 400 rpm, 37 °C for 72 h. The fed-batch reaction was initiated after intermittently feeding the reaction mixture composed of 250 mM (*R,S*)-FEE, 500 mM suβ-CD, and 600 units of lipase per 1 mmol (*R,S*)-FEE until a final (*R,S*)-FEE concentration of 1000 mM.

The (*S*)-, (*R*)-flurbiprofens and residual (*R,S*)-FEE were mixed with the same volume of chloroform, and then dissolved samples were analyzed by HPLC (Gilson Inc., France); RS-Tech TBB chiral column (0.46 cm × 25 cm), UV (250 nm) detector, *n*-hexane/methyl *tert*-butyl ether/acetate: 6/4/0.01 as the mobile phase, and a flow rate of 2.0 ml/min. The enantiomeric excess (ee) and conversion yield (*C*) were calculated as ee = [(*S*)-profen − (*R*)-profen]/[(*S*)-profen + (*R*)-profen], and *C* = [(*S*)-profen + (*R*)-profen]/[initial (*R,S*) − profen ethyl ester].

An amount of 25 ml of the fed-batch reaction mixture containing (*S*)-flurbiprofen and residual (*R*)-FEE as an inclusion complex with suβ-CD were extracted using 50 ml of various non-polar solvents. The (*S*)-flurbiprofen extracted in the non-polar solvent phase was then selectively dissolved using 50 ml of a 1 M sodium bicarbonate solution. Thereafter, the (*S*)-flurbiprofen selectively dissolved in the aqueous 1 M sodium bicarbonate solution was precipitated twice using 100 ml of 2-propanol.

The fed-batch reaction was carried out after the batch reaction that was nearly completed after 4 days as shown in Fig. 1, yielding 242 mM (*S*)-flurbiprofen with an enantiomeric excess and conversion yield of 0.98 and 0.48, respectively, similar levels obtained in our previous work [12]. After the batch reaction, the feeding mixture containing the immiscible (*R,S*)-FEE was intermittently fed twice up to a concentration of 1000 mM. An extremely high concentration of 480 mM optically active (*S*)-flurbiprofen, corresponding to 117.0 g/l, was then obtained after 16 days. The final ee value indicating the purity of the (*S*)-flurbiprofen, and conversion yield (*C*) indicating the degree of conversion also increased as high as 0.98 and 0.48, respectively, even under such an extremely high (*R,S*)-FEE concentration.

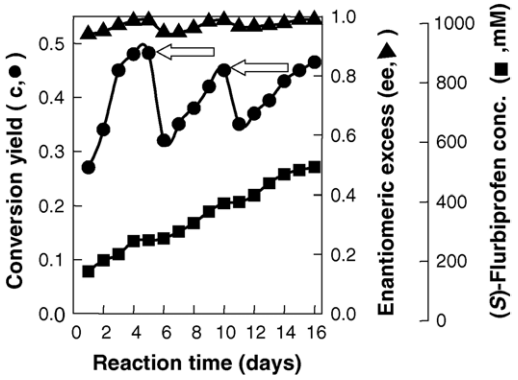

Fig. 1. Fed-batch production of (*S*)-flurbiprofen from (*R,S*)-FEE in a lipase-catalyzed dispersed aqueous phase reaction system induced by suβ-CD. Batch reaction was carried out using 500 mM (*R,S*)-FEE, 600 units of *C. rugosa* lipase per 1 mmol of (*R,S*)-FEE, and 1000 mM suβ-CD in 50 mM sodium phosphate buffer (pH 7.0) at 400 rpm, 37 °C for 4 days, and then the feeding mixture composed of 250 mM of (*R,S*)-FEE, 500 mM suβ-CD, and 600 units of lipase per 1 mmol (*R,S*)-FEE in 50 mM sodium phosphate buffer (pH 7.0) was intermittently fed. Feeding point (⟹), conversion yield (●), enantiomeric excess (△), concentration of (*S*)-flurbiprofen (■).

Fed-batch-wisely feeding seems to be a very useful method for the effective production of optically pure (*S*)-flurbiprofen.

(*S*)-Flurbiprofen and the residual (*R*)-FEE in the final reaction mixture existed in an inclusion complex form in the lipase-catalyzed dispersed aqueous phase reaction system induced by suβ-CD. The entrapped (*S*)-flurbiprofen was decomplexed from the cavity of the suβ-CD molecule using various non-polar solvents [6,14,15]. Fig. 2 compares the effects of various organic solvents according to the decomplexation yield of (*S*)-profen and residual (*R*)-FEE from the suβ-CD molecule. Ethyl acetate showed the most noticeable decomplexation activity with a decomplexation yield of 0.935, and 448.8 mM (*S*)-flurbiprofen was recovered from 480 mM (*S*)-flurbiprofen

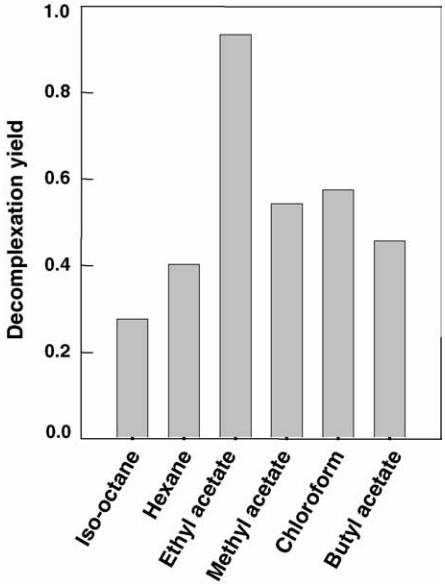

Fig. 2. Comparison of organic solvents on decomplexation of (*S*)-profen and residual (*R*)-FEE from inclusion complex with suβ-CD.

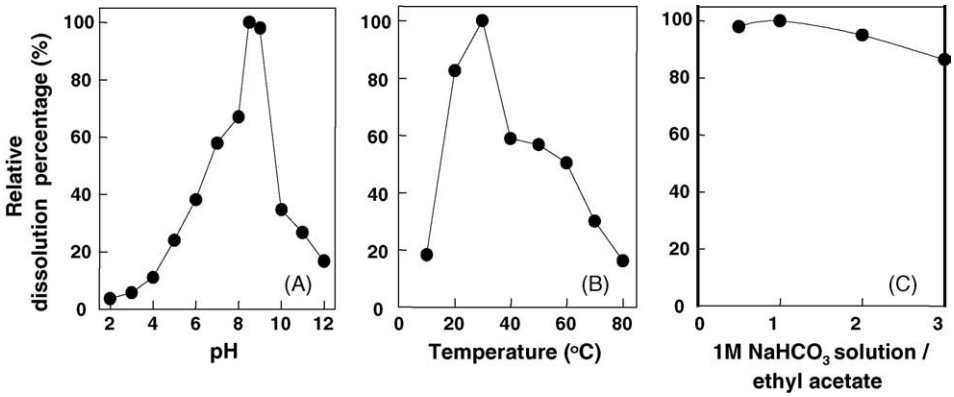

Fig. 3. Effects of dissolution pH, temperature and the amount of 1 M sodium bicarbonate on selective dissolution of (*S*)-flurbiprofen.

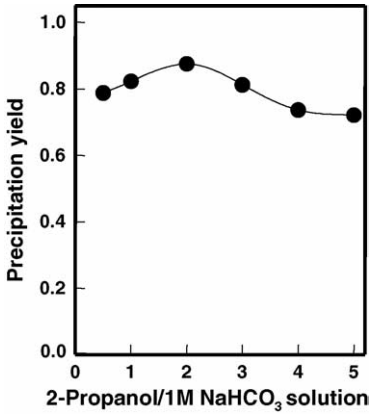

Fig. 4. Effect of mixing ratio between 2-propanol and 1 M sodium bicarbonate solution on precipitative purification of (*S*)-flurbiprofen.

fed-batch-wisely produced after decomplexation with ethyl acetate.

(*S*)-Flurbiprofen was then selectively dissolved using a 1 M sodium bicarbonate solution from the residual (*R*)-FEE in the upper ethyl acetate phase. As shown in Fig. 3, the optimal dissolution conditions were determined to be a pH of 8.5–9.0, a temperature of 30 °C, and a 1.0:1.0 volume ratio between the 1 M sodium bicarbonate solution and ethyl acetate. As a result, 428.2 mM (*S*)-flurbiprofen was recovered from 448.8 mM (*S*)-flurbiprofen dissolved in ethyl acetate, and the purity of the dissolved (*S*)-flurbiprofen was 95%.

(*S*)-Flurbiprofen dissolved using a 1 M sodium bicarbonate solution was selectively precipitated using 2-propanol to recover the optically pure (*S*)-flurbiprofen. The precipitation efficiency was also enhanced after adding a certain amount of 1 M sodium bicarbonate solution as shown in Fig. 4. Finally, an extremely high concentration of 420 mM, corresponding to 102.8 g/l of optically pure (*S*)-flurbiprofen with a purity higher than 98% was recovered after three-step purification procedures. An integrated process combining the kinetic resolution using fed-batch reaction and simple three-step purification procedures seems to be potentially applicable for the industrials production of optically pure (*S*)-flurbiprofen.

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