0273-2289/97/6803—0135$10.00

Surfactant Effect on Enhancing (S)-Naproxen Prodrug Production from Racemic Naproxen by Lipase

CHUN-SHENG CHANG AND SHAU-WEI TSAI*

Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan, 70101, Republic of China

Received January 14, 1997; Accepted April 2, 1997

ABSTRACT

In the enantioselective esterification of racemic naproxen with 4-(2-hydroxyethyl) morpholine by Lipase MY in organic solvents, a productivity improvement of the desired (S)-naproxen ester from 0.42 to 0.72 m*M* at the reaction time of 130 h was observed, when the surfactant *bis* (2-ethylhexyl) sodium sulfosuccinate (AOT) was added in the reaction mixture. The presence of a small amount of exogenously added water dramatically activated the enzyme in AOT/cyclohexane-reversed micelles. Desorption of the surfactant molecule from the enzyme mass and solubilization of the enzyme into reversed micelles were used to elucidate an existing maximum of the initial rate of (S)-naproxen synthesis with the water content. Moreover, the effects of alcohol and surfactant concentration on the enzyme activity are reported.

Index Entries: Lipase; (S)-naproxen ester prodrug; esterification; surfactant; reversed micelles.

INTRODUCTION

Naproxen (2-[6-methoxy-2-naphthyl] propionic acid) belongs to nonsteroidal antiinflammatory drugs (NSAIDs) and is used in the treatment of painful and inflammatory rheumatic or certain nonrheumatic conditions. With a chiral center at the 2-position, (S)-naproxen is 28-fold more active than its antipode, and is now marketed in enantiomerically pure form *(1)*. Previous pharmacological studies of (S)-naproxen and other acidic

*Author to whom all correspondence and reprint requests should be addressed.

NSAIDs indicated that gastrointestinal (GI) side effects constitute the most frequent adverse reactions. These range in both severity and frequency from relatively mild to the more serious states of GI ulceration and hemorrhage (*2*). Therefore, developing their bioreversible derivatives in order to decrease the toxicity induced by the acid moiety of NSAIDs is needed.

Esterification of acidic NSAIDs is an effective way to prepare the prodrug, since the organism is rich in enzymes capable of hydrolyzing the resultant ester (*3*). With a careful selection of the alcohol moiety, the ester prodrug of NSAIDs can often provide comparable bioactivity to that of the parent drug (*2,4,5*).

In a previous report, an enzymatic process was developed to synthesize 4-morpholinoethyl (S)-naproxen ester from racemic naproxen by lipases in organic solvents (*6*). With a careful selection of lipase source (Lipase MY) and organic solvent (cyclohexane), a high enantiomeric ratio of 136 was obtained. Since naproxen solubility in cyclohexane is low (1.22 m*M*, 37°C), it is necessary to find another reaction medium to increase naproxen solubility and, hence, the desired (S)-ester productivity. Adding a surfactant to the organic solvent has been shown to effectively enhance naproxen solubility in isooctane (*7*). Therefore, this strategy is extended to increase the productivity of 4-morpholinoethyl (S)-naproxen ester prodrug with Lipase MY as the biocatalyst in organic solvents.

MATERIALS AND METHODS

Materials

Optical pure (S)-naproxen was purchased from Sigma (St. Louis, MO). Isooctane and cyclohexane of ACS grade from Merck (Darmstadt, Germany) were used without further purification. Lipase MY (triacylglycerol ester hydrolases, EC 3.1.1.3) from *Candida rugosa* (30 U/mg solid) was provided by Meito Sangyo (Nagoya, Japan). Other chemicals of analytical grade were commercially available as follows: 4-(2-hydroxyethyl) morpholine from Aldrich (Milwaukee, WI); and *bis*(2-ethylhexyl) sodium sulfosuccinate (AOT) from Sigma (St. Louis, MO).

Analysis

The esterification was monitor by an HPLC using a chiral column (Chiralcel OD, Daicel Chemical, Japan) capable of separating (R)- and (S)-naproxen and their esters without derivatization (*5*). The mobile phase was a v/v mixture of n-hexane:isopropanol:acetic acid (97:3:1), at a flow rate of 1 mL/min. UV detection at 270 nm was for quantification at 25°C.

Two mL of 25–300 m*M* AOT in cyclohexane or isooctane was added to an excess amount of naproxen at room temperature. The solution was held at 50°C for 1 h and then kept at 37°C overnight. The saturated

naproxen solubility was measured by removing the superstratum for HPLC analysis.

Twenty mL of 300 m*M* AOT in isooctane was added to an appropriate amount of 67 m*M* phosphate buffer, pH 7.0, giving W_o (molar ratio of water to AOT) of 5.6. Lipase MY of 7 mg/mL was added to the resultant reversed micelles and stirred by a magnetic stirrer at 37°C. A sample of 1 mL was removed and centrifuged at different time intervals. The supertratum was analyzed at 280 and 310 nm by using a Shimadzu UV-160 spectrophotometer to determine the lipase concentration in the reversed micelles.

Methods

To 0.6 m*M* of racemic naproxen and 8 m*M* 4-(2-hydroxyethyl) morpholine in 20 mL of pure cyclohexane (or 50 m*M* AOT/cyclohexane-reversed micelles) was added 7 mg/mL of Lipase MY. The resultant mixture was stirred with a magnetic stirrer at 37°C. Samples were removed for HPLC analysis at different time intervals. Similar experiments were carried out, except that 10 m*M* of the alcohol was added to 25 m*M* AOT/cyclohexane (or 25 m*M* AOT/isooctane)-reversed micelles in which different volumes of a 67 m*M* phosphate buffer, pH 7.0, were added to control W_o.

Experiments were also carried out in 25 m*M* AOT/cyclohexane reversed micelles with W_o = 2.8, with the alcohol concentration varying from 4 to 45 m*M*. In order to illustrate the improvement of (S)-ester productivity, higher naproxen concentrations in cyclohexane and 25 m*M* AOT/cyclohexane-reversed micelles were applied in the experiments.

RESULTS AND DISCUSSION

Figure 1 illustrates the variation of saturated naproxen solubility (S), with AOT concentration (AOT), in organic solvents at 37°C. A linear relationship with the slope of 4.8×10^{-2} in AOT/isooctane and 7.4×10^{-2} in AOT/cyclohexane-reversed micelles, respectively, was found. Since cyclohexane is less hydrophobic than isooctane, a higher enhancement of naproxen solubility in the former was found for any AOT concentration.

The time-courses of naproxen conversion, X, studied at various values of W_o in isooctane and cyclohexane, are presented in Fig. 2. Compared with the result in pure cyclohexane, Lipase MY in isooctane possesses the highest conversion at any specific time, and hence the initial rate. This was previously observed *(5)* when other solvents with less hydrophobicity were used as the reaction media. Although organic solvents can affect enzyme performance in various ways *(8)*, a quantitative prediction of the effect is still difficult. When 50 m*M* of AOT was added to cyclohexane in the absence of the buffer (W_o = 0), the enzyme activity and naproxen conversion greatly decreased. However, this unfavorable effect is relaxed

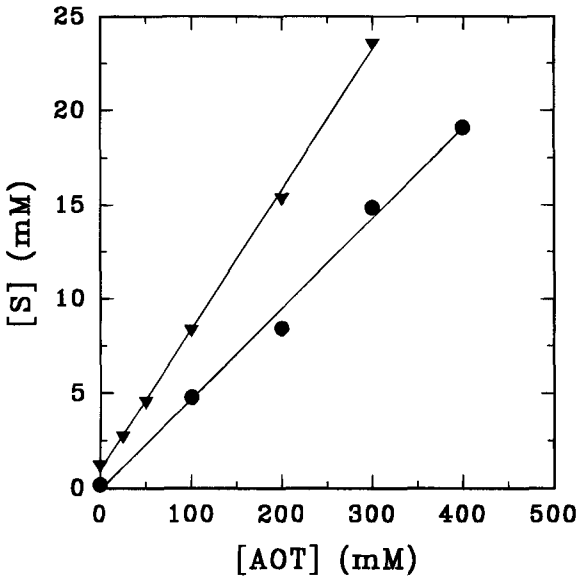

Fig. 1. Variation of saturated naproxen concentration with AOT concentration at 37°C in isooctane (●) and cyclohexane (▼).

by adding very small amounts of buffer into the system. After considering the higher naproxen solubility and final conversion, the system AOT/cyclohexane reversed micelles was selected as the reaction medium for further studies.

Figure 3 illustrates the variation of the initial rate of (S)-naproxen synthesis, V_A, at different W_o by using various AOT concentrations. Increasing the surfactant concentration from 25 to 100 m*M* at a fixed water content results in the decrease of initial esterification rate for (S)-naproxen. A noncompetitive inhibition by the surfactant to the enzyme has been proposed to elucidate this result in the esterification of naproxen, with trimethylsilyl methanol as an acyl acceptor in AOT/isooctane-reversed micelles *(7)*. This unfavorable surfactant inhibition on the enzyme activity could be compensated for by the enhancement of naproxen solubility, and might result in an increase of (S)-naproxen ester productivity, as shown below.

An asymmetric curve with a maximum initial rate at W_o values around 3.0 for 25 m*M* of AOT is shifted to that of 1.5 for 100 m*M* of AOT, as is also shown in Fig. 3. Two main mechanisms have been proposed to elucidate this behavior *(7)*. When the buffer is added and solubilizes into the inner core of the reversed micelles, the surfactant molecules, which exist as reversed micelles, free molecules, and those adsorbed on the enzyme, might redistribute. Therefore, the surfactant originally adsorbed on the lipase is reduced to decrease the surfactant inhibition, and enhances the enzyme activity. However, this favorable effect is offset by the quick solubilization of the lipase from the solvent into the water pool

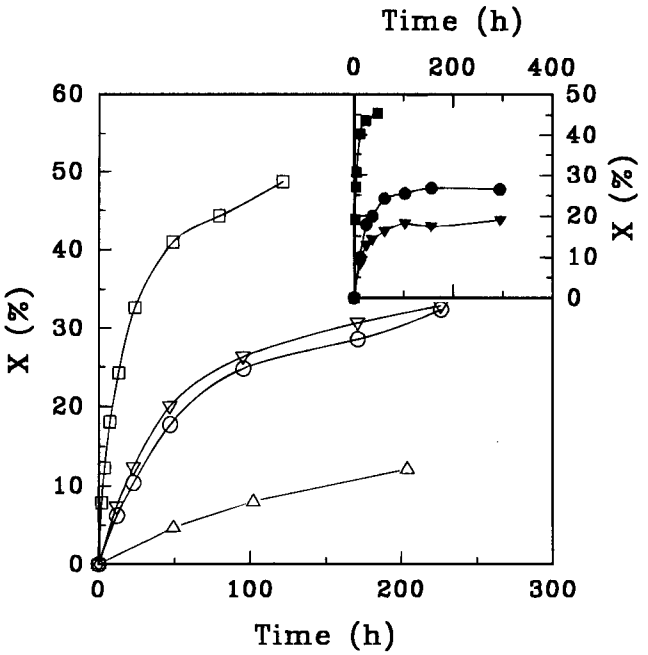

Fig. 2. Time-course of naproxen conversion as a function of W_0 in cyclohexane (hollow) and isooctane (filled) at 37°C. Unless specified, [AOT] = 25 m*M*, alcohol, enzyme, and naproxen concentrations are 10 m*M*, 7 mg/ml and 0.6 m*M*, respectively. (▽, ▼), W_0 = 1.44; (○, ●), W_0 = 2.78; (△), W_0 = 0 with [AOT] = 50 m*M*, alcohol concentration of 8 m*M*. (□), in cyclohexane with alcohol concentration of 8 m*M*, [AOT] = 0. (■), in isooctane with [AOT] = 0, alcohol, enzyme, and naproxen concentrations of 5.4 m*M*, 5 mg/mL, and 0.22 m*M*, respectively.

of reversed micelles, a process that could lead to deactivation of the enzyme. A typical example is shown in Fig. 4, in which more than 60% of the original suspended lipase was solubilized into AOT/isooctane-reversed micelles within 10 min. Therefore, as W_0 increases at lower water contents, desorption of AOT from the enzyme mass is dominant to reduce the surfactant inhibition and increase the enzyme activity. When W_0 is greater than the optimal value, the solubilization of lipase into reversed micelles is more important to decrease the initial rate for (S)-naproxen.

The variation of the initial rates for (R)- and (S)-naproxen synthesis of varying alcohol concentration (C) is shown in Fig. 5. Maximum initial rates for both enantiomers were found at the alcohol concentration around 19 m*M* in 25 m*M* AOT/cyclohexane-reversed micelles. This implies that the alcohol might have acted as an enzyme inhibitor. However, an optimal alcohol concentration around 8 m*M* in pure cyclohexane is also presented in the figure. This large difference of optimal alcohol concentrations in both systems might be caused by the solubilization of alcohol into reversed micelles. Because the partition coefficient of 4-(2-hydroxyethyl) morpholine between a buffer solution of pH 7.0 and cyclohexane at 37°C was measured as 310, we have calcu-

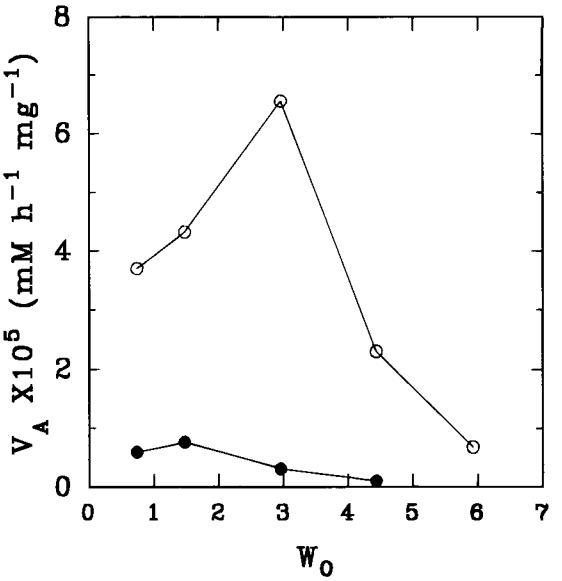

Fig. 3. Variation of initial rate of (S)-naproxen as a function of W_0 in AOT/cyclohexane reversed micelles at 37°C: alcohol, enzyme, and naproxen concentrations of 10 m*M*, 7 mg/mL and 0.6 m*M*, respectively. (○), [AOT] = 25 m*M*; (●), [AOT] = 100 m*M*.

lated the effective alcohol concentration in cyclohexane and presented it in the figure, in which an optimal effective alcohol concentration around 5 m*M* was demonstrated. This is comparable with that found in pure cyclohexane.

The improvement of the present reaction system is better illustrated by comparing the time-courses of (R)- and (S)-naproxen conversions and (S)-naproxen ester concentration in cyclohexane and AOT/cyclohexane-reversed micelles. Preliminary results are represented in Fig. 6, in which a nearly saturated naproxen concentration, with the optimal alcohol concentration of 19 m*M* (in Fig. 5) for each reaction system, was used. As expected, a lower conversion for each enantiomer in 25 m*M* AOT/cyclohexane-reversed micelles was obtained because of the surfactant inhibition. However, when AOT was added, (S)-naproxen ester was improved from 0.42 m*M* to 0.72 m*M* at the reaction time of 130 h, when the enantiomeric excesses of the product in both systems, i.e., 0.83 and 0.85, were almost the same.

CONCLUSIONS

The surfactant effect on the lipase-catalyzed enantioselective synthesis of (S)-naproxen ester prodrug at 37°C in organic solvents was investigated. Naproxen solubility in isooctane or cyclohexane is improved by adding AOT as the surfactant. The low enzyme activity observed because of surfactant inhibition was relaxed by adding a small amount of water. The unfavorable surfactant inhibition on the enzyme activity is compensated for by the

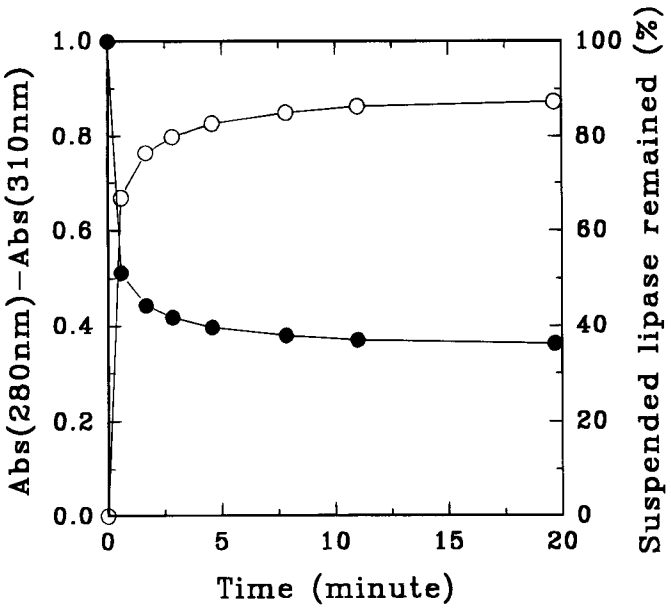

Fig. 4. Time-course UV absorptivity (○) and suspended lipase remained in isooctane (●) at 37°C, [AOT] = 300 mM, W_o = 5.56 and enzyme concentration of 7 mg/mL.

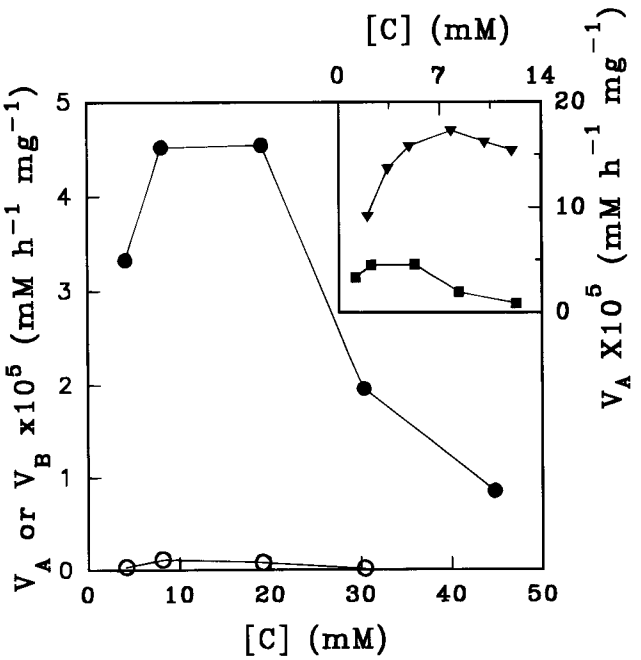

Fig. 5. Variation of initial rates of (R)-naproxen, V_B (hollow), and (S)-naproxen, V_A (filled), at different alcohol concentrations [C], at 37°C: enzyme and naproxen concentrations of 7 mg/mL and 0.6 mM, respectively. (▼), in pure cyclohexane; (○, ●, ■), in 25 mM AOT/cyclohexane-reversed micelles with W_o = 2.78. [C] in (■) is the effective alcohol concentration.

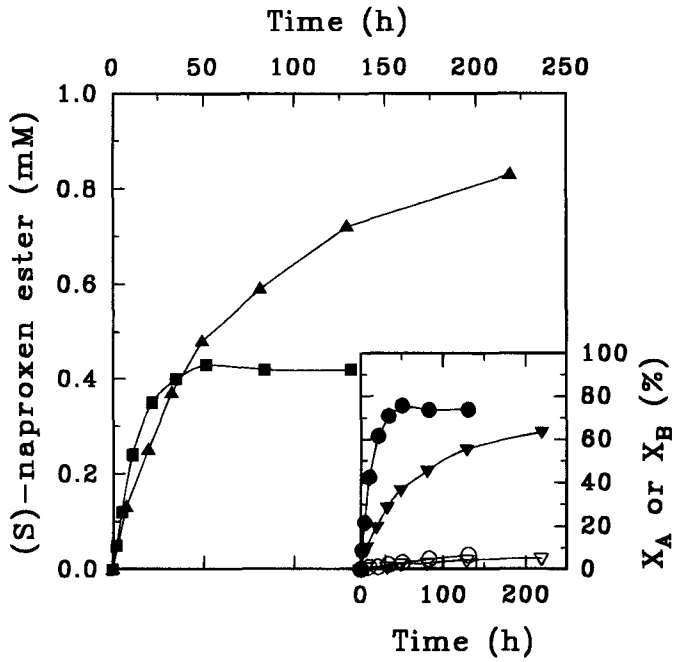

Fig. 6. Time-courses of (R)-naproxen conversion, X_B (○), (S)-naproxen conversion, X_A (●), and (S)-naproxen ester concentration (■), with enzyme concentration of 7 mg/mL, [C] = 8 m*M*, and naproxen concentration of 1.3 m*M* in cyclohexane, respectively. (▼) for X_A, (▽) for X_B, and (▲) for (S)-naproxen ester concentration in 25 m*M* AOT/cyclohexane-reversed micelles, with W_o = 1.44, [C] = 19 m*M*, and naproxen concentration of 2.6 m*M*.

enhancement of naproxen solubility, and results in an increase of (S)-naproxen ester productivity, without any loss of enantiomeric excess of the product.

ACKNOWLEDGMENT

Financial support from the Chinese National Science Council with Grant No. NSC-84-2214-E-006-026 is appreciated.

REFERENCES

1. Todd, P. A. and Clissold, S. P. (1990), *Drugs*, **40**, 91–137.
2. Shanbhag, V. R., Crider, A. M., Harpalani, R. A., and Dick, R. M., (1992), *J. Pharm. Sci.*, **81**, 149–154.
3. Bundgaard, H. (1985), *Design of Prodrugs*, Elsevier, Amsterdam.
4. Nielsen, N. M. and Bundgaard, H., (1988), *J. Pharm. Sci.* **77**, 285–298.
5. Tammara, V. K., Narurkar, M. M., Crider, A. M., and Khan, A. M. (1993), *Pharm. Res.*, **10**, 1191–1199.
6. Chang, C. S. and Tsai, S. W., (1997), *Enzyme Microb. Technol.*, **20**, 635–639.
7. Tsai, S. W., Lu, C. C., and Chang, C. S., (1996), *Biotech. and Bioeng.*, **51**, 148–156.
8. Halling, P. J., (1994), *Enzyme Microb. Technol.*, **16**, 178–206.