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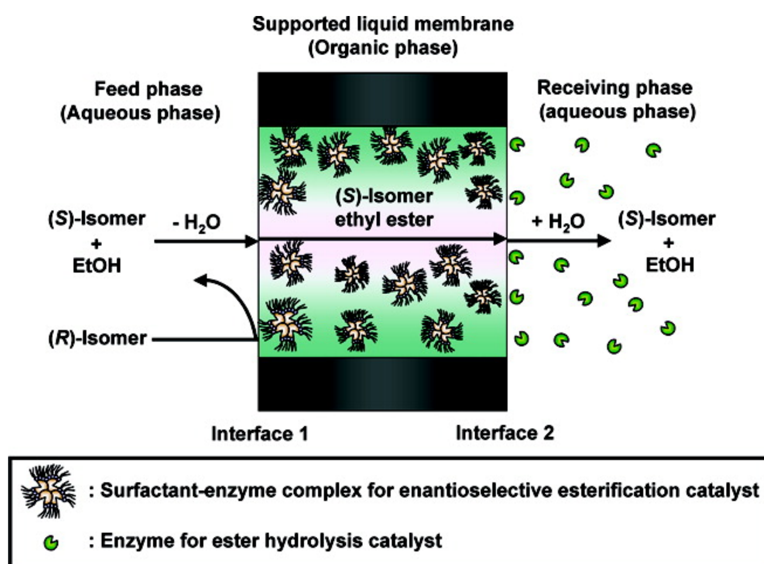

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Highly Enantioselective Separation Using a Supported Liquid Membrane Encapsulating Surfactant−Enzyme Complex

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There is an increasing demand for optically pure enantiomers in the chemical industry.[1] Organic acids and amino acids represent a large portion of this market, but for these useful organic compounds, only one enantiomer is known to be normally biologically active. Many researchers have attempted the separation of optically active compounds.[2] Some enzymes, such as lipase and protease, catalyze highly enantioselective hydrolysis or esterification of drugs and amino acids. By employing the enantioselectivity of these enzymes, optical resolutions of a number of racemic mixtures have been reported.[3] These studies demonstrated the selective production of optically pure compounds from racemic mixtures; however, this enzymatic technique requires further processing (crystallization, solvent extraction, etc.) to separate the target enantio-derivative from another enantiomer.

Supported liquid membranes (SLMs), in which the organic liquid is entrapped in a porous membrane, have been widely studied as a selective separation technique.[4] Application of this technique has, however, been limited mainly to the separation of metal ions and amines to utilize the crown ethers. Rethwisch et al. and we previously demonstrated that lipase-catalyzed reactions (esterification and hydrolysis) drove highly selective separation of organic acids through the bulk liquid membrane and SLM, in which the selectivity of the organic acids separation was based on the substrate specificity of the lipases.[5] In the previous system, the transport efficiency for a targeted organic acid and the enantioselectivity were not satisfactory; we presumed that the low transport efficiency and the low enantioselectivity were due to the low esterification activity of native lipase deposited in the aqueous phase.

To date, several research groups, along with ours, have demonstrated that a surfactant−enzyme complex, which was soluble in organic solvents, effectively catalyzed esterification reaction in organic media.[6] In the present study, we developed a novel SLM encapsulating the surfactant−enzyme complex in the liquid membrane phase and succeeded in an efficient and highly enantioselective separation for the optically active compounds (*S*)-ibuprofen and L-phenylalanine from their racemic mixtures.

Figure 1 gives a schematic diagram of the enantioselective separation system for the racemic mixtures through the SLM encapsulating the surfactant−enzyme complex. The surfactant−enzyme complex can be solubilized in the thin organic membrane and effectively catalyzes the esterification reaction in the thin film. The surfactant−enzyme complex is good at catalyzing enantioselective esterification in the liquid membrane phase, but another enzyme is used as an ester hydrolysis catalyst in the receiving phase. Therefore, the (*S*)-isomer is selectively esterified by the surfactant−enzyme complex at interface 1 in the SLM phase, and the resulting ethyl ester of (*S*)-isomer dissolves into the organic phase of the SLM and diffuses across the SLM. At interface 2 in the receiving phase, another enzyme catalyzes the ester hydrolysis to produce the initial (*S*)-isomer and ethanol, which are water-soluble. Thus,

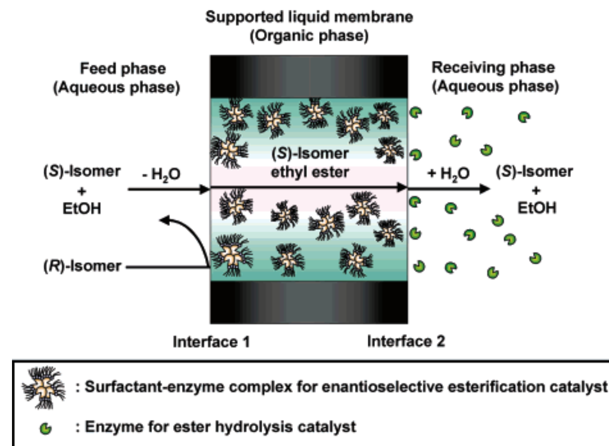

Figure 1. Concept of enantioselective separation of racemic mixtures through the SLM encapsulating the surfactant−enzyme complex.

the (*S*)-isomer is selectively transported to the receiving phase through the SLM, based on the enantioselectivity of the enzymes.

The SLM encapsulating the surfactant−enzyme complex[7] was prepared by immersing a hydrophobic poly(propylene) film (Celgard 2500; the thickness of the film was 25 μm, and the maximum pore size was 0.2×0.05 μm) into isooctane containing the surfactant−enzyme complex. Enantioselective transport experiments through the SLM were performed at 37 °C using a pair of glass cells (each cell had a volume of 55 mL and a 5 cm^2 cross-section). The SLM encapsulating the surfactant−enzyme complex separated the two aqueous phases. The feed phase consisted of McIlvaine buffer (pH 6.3) containing 10 mM racemic substrate and ethanol. The receiving phase consisted of McIlvaine buffer (pH 6.3) containing hydrolysis biocatalyst. The concentrations of (*S*)- and (*R*)-isomer of substrates in the feed and receiving phase were determined by HPLC analysis.

Figure 2 depicts the enantioselective transport of (*S*)-ibuprofen through the SLM encapsulating surfactant−lipase complex. The surfactant−lipase CRL (lipase from *Candida rugosa*) complex was encapsulated in the SLM, and native lipase PPL (lipase from porcine pancreas) was dissolved in the receiving phase (see Figure 2 caption for more detail). The (*S*)-ibuprofen concentration in the receiving phase increased with time, with that in the feed phase correspondingly decreasing. In contrast, the (*R*)-ibuprofen concentration in the receiving phase did not notably increase, while that in the feed phase did not decrease. A control experiment performed without the lipases resulted in no ibuprofen transport through the SLM, because ibuprofen was insoluble in isooctane, indicating that the lipase-catalyzed reactions drove the transport of (*S*)-ibuprofen through the SLM, as shown in Figure 1. A high enantiomeric excess (ee = 91 (%)) value for (*S*)-ibuprofen was obtained at the end of the operation (48 h). These results indicate that the enantioselectivity of the lipases induced the difference between the transport behavior

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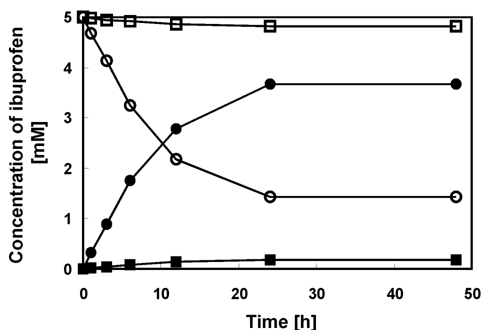

Figure 2. Lipase-facilitated transport of (*S*)-ibuprofen through the SLM. (●) (*S*)-Ibuprofen in the receiving phase, (○) (*S*)-ibuprofen in the feed phase, (■) (*R*)-ibuprofen in the receiving phase, (□) (*R*)-ibuprofen in the feed phase. The feed phase consisted of McIlvaine buffer (pH 6.3) containing 10 mM racemic ibuprofen and 50 vol % ethanol. The SLM encapsulated 5 mg/mL CRL complex. The receiving phase consisted of McIlvaine buffer (pH 6.3) containing 8 mg/mL native PPL. Dioleyl-L-glutamate ribitol was used as the surfactant for the surfactant−lipase complex.

of (*S*)- and (*R*)-ibuprofen through the SLM. Indeed, the lipase CRL has been reported to be a useful biocatalyst for the enantioselective esterification reaction of (*S*)-ibuprofen.[8] The effect of different kinds of lipase on the enantioselective transport of (*S*)-ibuprofen was examined. In these results, the maximum ee (91%) was obtained when the surfactant−CRL complex was used in the SLM. The maximum permeate flux of (*S*)-ibuprofen (0.58 [mol/(m^2•h)]) was obtained when using 5 mg/mL surfactant−CRL complex in the SLM and 8 mg/mL PPL in the receiving phase. In our previous lipase-facilitated SLM, a large quantity of lipases (total 2750 mg (CRL = 1650 mg, PPL = 1100 mg) was required to transport organic acids.[5c,d] In the present study, the net amount of lipase CRL was just 1 mg, which is 1/1650 that of our previous system, and the amount of lipase PPL was 440 mg, which is 2/5 that of our previous system. The high esterification activity of the surfactant−CRL complex in the SLM would contribute to drastically decreasing the amounts of lipases.

Figure 3 shows the selective transport of L-phenylalanine through the SLM encapsulating the surfactant−α-chymotrypsin complex. The α-chymotrypsin complex was entrapped in the SLM phase and also dispersed in the receiving phase (see Figure 3 caption for more detail). The L-phenylalanine concentration in the receiving phase increased with time. On the other hand, D-phenylalanine was not transported through the SLM. It is noteworthy that the α-chymotrypsin-facilitated SLM system achieved ee >99% for L-phenylalanine by the end of the operation (48 h) and a maximum permeate flux of L-phenylalanine (0.18 [mol/(m^2•h)]). Phenylalanine was not transported through the SLM at all in a control experiment without the surfactant−α-chymotrypsin complex. α-Chymotrypsin has also been studied as an enantioselective biocatalyst for various amino acids,[9] suggesting that the α-chymotrypsin-catalyzed reactions drove the enantioselective transport of L-phenylalanine based on the enantioselectivity of α-chymotrypsin. The permeate flux of L-phenylalanine was less than that of (*S*)-ibuprofen; differences in this transport behavior could be explained by the difference in the solubilities of each esterified substrate in the SLM phase[10] and differences between enzymatic activities in the organic solvent and at the interfaces.[11]

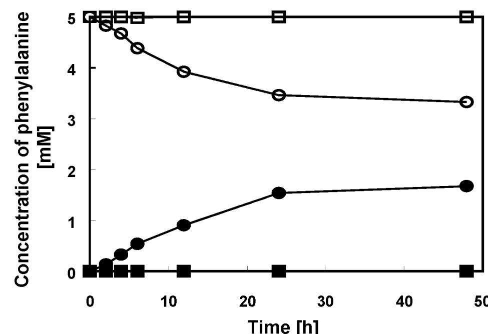

Figure 3. α-Chymotrypsin-facilitated transport of L-phenylalanine through the SLM. (●) L-Phenylalanine in the receiving phase, (○) L-phenylalanine in the feed phase, (■) D-phenylalanine in the receiving phase, (□) D-phenylalanine in the feed phase. The feed phase consisted of McIlvaine buffer (pH 6.3) containing 10 mM racemic phenylalanine and 40 vol % ethanol. The SLM encapsulated 5 mg/mL α-chymotrypsin complex. The receiving phase consisted of McIlvaine buffer (pH 6.3) containing 0.5 mg/mL α-chymotrypsin complex. The α-chymotrypsin complex was prepared with the same surfactant employed for the surfactant−lipase formulation.

In conclusion, the SLM encapsulating the surfactant−enzyme complex enabled highly enantioselective separation of racemic ibuprofen and phenylalanine. It can be envisioned that the arrangement of appropriate enzymes in the SLM system will allow enantioselective separations of various useful organic compounds.

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References

(1) Crosby, J. *Tetrahedron* **1991**, *47*, 4789.
(2) (a) Lee, S. B.; Mitchell, D. T.; Trofin, L.; Nevanen, T. K.; Söderlund, H.; Martin, C. R. *Science* **2002**, *296*, 2198. (b) Chen, C.; Wu, S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2812.
(3) (a) Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc*. **1985**, *107*, 7072. (b) Lalonde, J. J.; Govardhan, C.; Khalaf, N.; Martinez, A. G.; Visuri, K.; Margolin, A. L. *J. Am. Chem. Soc.* **1995**, *117*, 6845.
(4) Reviews: (a) Jönsson, J. Å.; Mathiasson, L. *Trends Anal. Chem*. **1999**, *18*, 318. (b) Jönsson, J. Å.; Mathiasson, L. *J. Chromatogr., A*. **2000**, *902*, 205. (c) de Gyves, J. de Miguel, E. R. *Ind. Eng. Chem. Res*. **1999**, *38*, 2182.
(5) (a) Rethwisch, D. G.; Subramanian, A.; Yi, G.; Dordick, J. S. *J. Am. Chem. Soc*. **1990**, *112*, 1649. (b) Rethwisch, D. G.; Parida, S.; Yi, G.; Dordick, J. S. *J. Membr. Sci*. **1994**, *95*, 83. (c) Miyako, E.; Maruyama, T.; Kamiya, N.; Goto, M. *J. Biosci. Bioeng.* **2003**, *96*, 370. (d) Miyako, E.; Maruyama, T.; Kamiya, N.; Goto, M. *Chem. Commun*. **2003**, 2926.
(6) (a) Okahata, Y.; Ijiro, K. *J. Chem. Soc., Chem. Commun.* **1988**, 1392. (b) Noda, S.; Kamiya, N.; Goto, M. *Biotechnol. Lett.* **1997**, *19*, 307. (c) Kamiya, N.; Goto, M. *Biotechnol. Prog*. **1997**, *13*, 488. (d) Paradkar, V. M.; Dordick J. S. *J. Am. Chem. Soc.* **1994**, *116*, 5009.
(7) (a) Goto, M.; Matsumoto, M., Kondo, K.; Nakashio, F. *J. Chem. Eng. Jpn*. **1995**, *11*, 893. (b) Goto, M.; Noda, S.; Kamiya, N.; Nakashio, F. *Biotechnol. Lett.* **1996**, *18*, 839. (c) Goto, M.; Sumura, H.; Abe, K.; Nakashio, F. *Biotechnol. Tech.* **1995**, *9*, 101.
(8) Klibanov, A. M. *Acc. Chem. Res.* **1990**, **23**, 114.
(9) (a) Oshima, T.; Inoue, K.; Furusaki, S.; Goto, M. *J. Membr. Sci.* **2003**, *217*, 87. (b) Nakamoto, Y.; Karube, I.; Kobayashi, I.; Nishida, M.; Suzuki, S. *Arch. Biochem. Biophys.* **1979**, *193*, 117.
(10) (a) Branco, L. C.; Crespo, J. G.; Afonso, C. A. M. *Angew. Chem., Int. Ed.* **2002**, *41*, 2771. (b) Branco, L. C.; Crespo, J. G.; Afonso, C. A. M. *Chem. Eur. J.* **2002**, *17*, 3865.
(11) (a) Narayan, V. S.; Klibanov, A. M. *Biotechnol. Bioeng*. **1993**, *41*, 390. (b) Filho, M. V.; Stillger, T.; Müller, M.; Liese, A.; Wandrey, C. *Angew. Chem., Int. Ed.* **2003**, *42*, 2993. (c) Maruyama, T.; Nakajima, M.; Ichikawa, S.; Nabetani, H.; Furusaki, S.; Seki, M. *J. Am. Oil Chem. Soc.* **2000**, *77*, 1121. (d) Maruyama, T.; Nakajima, M.; Seki, M. *J. Biosci. Bioeng*. **2001**, *92*, 242.

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