A Supported Liquid Membrane Encapsulating a Surfactant–Lipase Complex for the Selective Separation of Organic Acids

Eijiro Miyako, Tatsuo Maruyama,* Noriho Kamiya, and Masahiro Goto*[a]

Abstract: We have developed a novel, lipase-facilitated, supported liquid membrane (SLM) for the selective separation of organic acids by encapsulating a surfactant–lipase complex in the liquid membrane phase. This system exhibited a high transport efficiency for 3-phenoxypropionic acid and enabled the selective separation of organic acids due to the different solubilities of the acids in the organic phase and the variable substrate specificity of the surfactant–lipase complex in the liquid membrane phase. We found that various parameters, such as the amount of surfactant–lipase complex in the SLM, the lipase concentration in the receiving phase, and the ethanol concentration in the feed phase, affected the transport behavior of organic acids. The optimum conditions were 5 g L^{-1} of the surfactant–CRL complex in the SLM (CRL = lipase from *Candida rugosa*), 8 g L^{-1} of PPL in the receiving phase (PPL = lipase from porcine pancreas), and an ethanol concentration of 50 vol%. Furthermore, we achieved high enantioselective transport of (*S*)-ibuprofen attributable to the enantioselectivity of the surfactant–CRL complex.

Keywords: chiral resolution · enzyme catalysis · lipases · organic acids · supported liquid membranes

Introduction

A supported liquid membrane (SLM) facilitates the selective separation of a target molecule by either exploiting the solubility differences between solutes in the liquid membrane phase or by the specific interaction between a carrier and the target molecule.[1] Application of the SLM technique has been limited mainly to the separation of metal ions, although the separation of amines, antigens, and sugars has also been reported.[1c,2] We previously used an SLM coupled with lipase-catalyzed reactions to achieve selective transport of organic acids through the SLM, in which the selectivity was assumed to be due to the substrate specificity of the lipase.[3] In our previous SLM systems, an organic acid was esterified by a lipase in the feed aqueous phase. The resulting ethyl ester then dissolved in the liquid membrane phase, diffused across the SLM, and was hydrolyzed by a different lipase in the receiving aqueous phase. Unfortunately, the transport efficiencies for organic acids were unsatisfactory, even when a large amount of lipase was used for esterification. This is presumably due to the low esterification activity in the feed aqueous solution.

In the last decade, great progress has been made in the study of nonaqueous enzymology, for example, the catalysis of synthetic reactions in organic solvents. In particular, a surfactant (dioleyl-L-glutamate ribitol ($2C_{18}\Delta^9$GE))–lipase (from *Candida rugosa*) complex, which is soluble in organic solvents, effectively catalyzed esterification and interesterification in organic media.[4–6] Therefore, we encapsulated the surfactant–lipase complex in the liquid membrane phase of the SLM. This complex is expected to catalyze the substrate-specific or enantioselective esterification of various organic acids within the thin liquid membrane film, resulting in improved transport of the organic acid through the SLM. We recently demonstrated an SLM that encapsulated surfactant–enzyme (lipase and α-chymotrypsin) complexes and that could achieve highly enantioselective transport of (*S*)-ibuprofen and L-phenylalanine.[6] In the present study, the selective separation of organic acids and the enantioselective transport of (*S*)-ibuprofen by means of the SLM encapsulating the surfactant–lipase complex are investigated in detail, and important parameters affecting transport efficiency are discussed.

Figure 1a shows a schematic diagram of organic acid transport through the SLM encapsulating the surfactant–lipase complex. R_ACOOH, R_BCOOH, EtOH, and R_A-COOEt represent the organic acid transported, the organic

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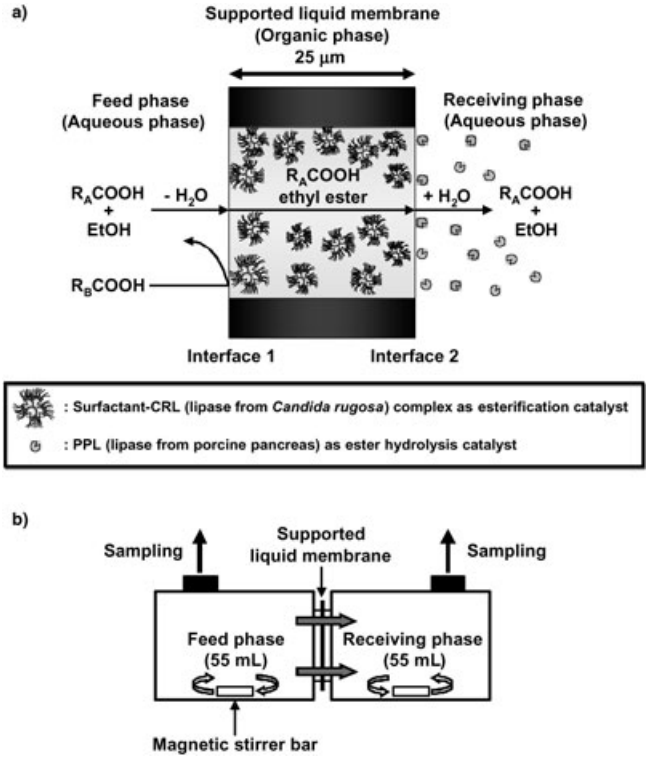

Figure 1. a) Schematic concept of the lipase-facilitated transport of a targeted organic acid through the SLM encapsulating the surfactant–lipase complex. b) Schematic illustration of the SLM cell, in which the SLM separates the aqueous phases. The apparatus was maintained at 37 °C in a thermostatically controlled water bath.

acid not transported, the carrier ethanol, and the resulting ethyl ester, respectively. The surfactant–CRL complex (CRL = lipase from *Candida rugosa*) acts as a biocatalyst for esterification in the organic phase (at Interface 1), whereas lipase PPL (from porcine pancreas) in the receiving phase hydrolyzes the ester (Interface 2). As a result, R_ACOOH is esterified by the surfactant–CRL complex at Interface 1, R_ACOOEt partitions into the organic phase of the SLM, and then diffuses across the SLM. (R_BCOOH is not esterified by the surfactant–CRL complex because the latter does not recognize R_BCOOH as a substrate.) At Interface 2 in the receiving phase, PPL catalyzes the hydrolysis of R_ACOOEt to ethanol and the original R_ACOOH, both of which are soluble in water. The overall result is that R_ACOOH is transported through the SLM.

Results and Discussion

Transport of 3-phenoxypropionic acid (3-PPA) through the SLM encapsulating the surfactant–lipase complex: We previously developed an SLM in which lipases CRL and PPL, dissolved in the feed and receiving aqueous phases, respectively, facilitated the transport of organic acids through the SLM.[3,7] CRL served as an esterification catalyst in the feed phase to produce the ethyl esters of the organic acids.[8] Unfortunately, the esterification activity of CRL is very low in an aqueous phase and resulted in the unsatisfactory permeate flux of organic acids through the SLM.

In the present study, we employed a surfactant–CRL complex in the liquid membrane phase as an esterification biocatalyst instead of native CRL in the feed aqueous phase. The surfactant–CRL complex can be solubilized in organic solvents and exhibits high esterification activity in organic media.[4,5s,6] The encapsulation of this complex in the liquid membrane phase of the SLM was expected to effectively esterify a targeted organic acid at Interface 1 and to initiate its transport through the SLM (Figure 1a). Figure 2 shows the

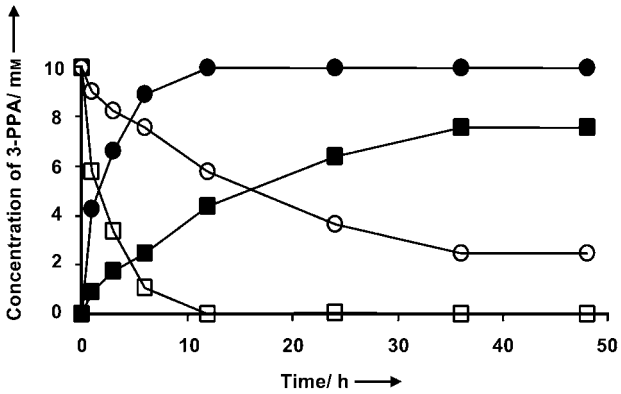

Figure 2. Lipase-facilitated transport of 3-PPA through the SLM encapsulating the surfactant–lipase complex (concentration of 3-PPA in the receiving (●) and feed (○) phases) and through the lipase-facilitated SLM developed previously[3a] (concentration of 3-PPA in the receiving (■) and feed (□) phases).

lipase-facilitated transport of 3-phenoxypropionic acid (3-PPA) through the SLM encapsulating the surfactant–CRL complex, and through the previously reported lipase-facilitated SLM.[3a] In the case of the CRL complex, the concentration of 3-PPA increased in the receiving phase over time and decreased simultaneously in the feed phase. After 12 h, the 3-PPA concentration in the receiving phase had reached a plateau (about 10 mM). Notably, a high initial permeate flux of 3-PPA (2.97 $mol\,m^{-2}h^{-1}$) was obtained and all of the 3-PPA had been transported from the feed phase to the receiving phase within 12 h. Control experiments, in which lipases were absent in either the liquid membrane or receiving phases, resulted in no 3-PPA transport through the SLM in either case. These permeation experiments were performed in duplicate; we obtained almost the same results and confirmed their reproducibility. These results indicated that the transport of 3-PPA through the SLM encapsulating the surfactant–CRL complex was enabled by lipase-catalyzed reactions, as shown in Figure 1. In contrast, for the previously reported lipase-facilitated SLM, the concentration of 3-PPA in the receiving phase increased over time and reached a maximum of around 8 mM. The initial permeate flux of the 3-PPA was 0.81 $mol\,m^{-2}h^{-1}$, which is less than

one third of the value recorded for the SLM encapsulating the lipase complex. We have already demonstrated that the surfactant–lipase complex exhibits high esterification activity in organic solvents.[4,5a,6] The notably higher permeate flux of 3-PPA is, therefore, due to the high esterification activity of the surfactant–lipase complex in the organic liquid membrane phase.

Effect of the amount of lipase on the permeate flux of 3-PPA: Because the lipase-catalyzed reactions drive the permeation of 3-PPA through the SLM, it is expected that the permeate flux of 3-PPA is influenced by the amount of lipase in the liquid membrane and receiving phases. Figure 3a and b show the effects of increasing the amounts of

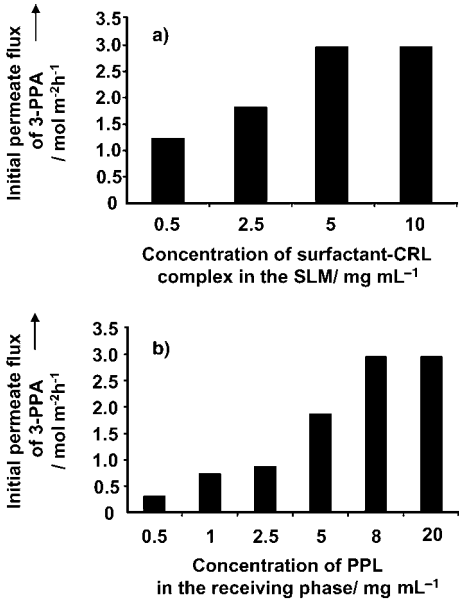

Figure 3. Effect of concentration of biocatalyst on the transport of 3-PPA. a) Variable concentration of surfactant–lipase complex in the SLM. The concentration of PPL in the receiving phase was fixed at 8 gL^{-1}. b) Variable concentration of PPL in the receiving phase. The concentration of the surfactant–CRL complex in the SLM was fixed at 5 gL^{-1}.

surfactant–CRL complex and PPL in the SLM and receiving phase, respectively. The maximum permeate flux of 3-PPA (2.97 mol$m^{-2}h^{-1}$) was obtained when the concentration of the surfactant–CRL complex was greater than 5 gL^{-1} and the concentration of PPL was 8 gL^{-1}, or when the concentration of PPL was greater than 8 gL^{-1} and the concentration of the surfactant–CRL complex was 5 gL^{-1}. Results for the previously reported lipase-facilitated SLM revealed that a large quantity of the lipases (CRL = 1650 mg, PPL = 1100 mg, total 2750 mg) was required to effectively transport 3-PPA. In the present study, the net amount of CRL was 1 mg, less than $^{1}/_{1000}$ of that used in our previous system, and the amount of PPL was 440 mg, approximately $^{2}/_{5}$ of that used in our previous system. The high esterification activity of the surfactant–CRL complex in the SLM contributes to the dramatic decrease in the amount of lipases required.

Effect of ethanol concentration on the transport of 3-PPA through the SLM: The transport of 3-PPA through the SLM was initiated by the esterification of 3-PPA, catalyzed by the surfactant–lipase complex. The effect of the concentration of ethanol in the feed phase on the transport behavior of 3-PPA was investigated (Figure 4a). It was observed that, as

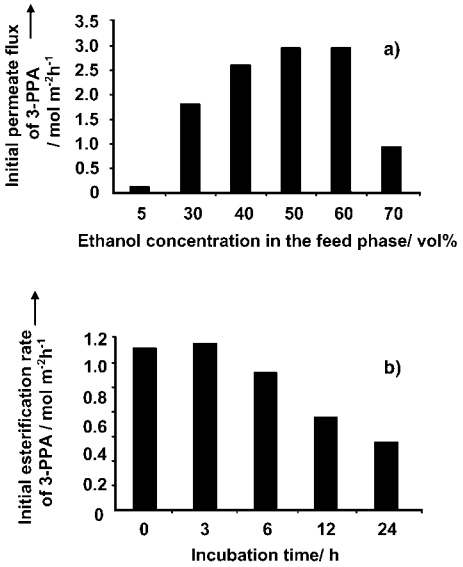

Figure 4. a) Effect of ethanol concentration in the feed phase on the initial permeate flux of 3-PPA. b) Stability of the surfactant–lipase CRL complex in an isooctane–water biphasic system containing a high concentration of ethanol.

ethanol concentration increased, the initial permeate flux of 3-PPA increased. The maximum permeate flux was obtained at 50 and 60 vol% ethanol in the feed phase. Further additions of ethanol reduced the permeate flux. The esterification of 3-PPA by the surfactant–CRL complex at Interface 1 plays an essential role in the transport of 3-PPA through the SLM. However, in the presence of abundant water, lipase predominantly catalyzes hydrolysis rather than esterification. Therefore, such a high ethanol concentration (50 or 60 vol%) in the feed phase was required for the effective transport of 3-PPA. An ethanol concentration greater than 60 vol% probably induces lipase denaturation, leading to a decrease in the permeate flux.[9] Notably, the SLM was stable for 90 h, even in the presence of such a high ethanol concentration.

Next, we studied the stability of the surfactant–CRL complex in an isooctane–water biphasic system containing a high concentration of ethanol (Figure 4b). This system consisted of an aqueous buffer containing ethanol (50 vol%), and isooctane containing the surfactant–CRL complex. At each incubation time, 3-PPA was added to the solution and the activity of the surfactant–CRL complex was measured (see Experimental Section). The activity of the complex decreased as incubation time increased; after 12 h incubation, the activity was approximately half of that measured at 0 h incubation. These results demonstrate that the surfactant–

CRL complex in the organic phase was gradually denatured in the SLM, which is inevitable in the present system. It will be necessary to overcome the denaturation of the enzyme before a continuous SLM system with high performance can be developed.

The transport behavior of various organic compounds: By employing this SLM system, the transport behavior of various organic compounds was investigated (Figure 5). The initial permeate fluxes of 3-PPA and 4-phenoxybutyric acid (4-PBA) were higher than those of other organic compounds, such as 2-phenylpropionic acid (2-PPA) and 2-phenoxybutyric acid (2-PBA), which were also transported but at much lower rates. Mandelic acid (MA), 2-amino-2-phenyl butyric acid (2-A-2-PBA), phenylalanine (Phe), and tryptophan (Trp) were not transported through the SLM at all. The differences in the transport of organic compounds may be due to the substrate specificity of lipases, which are restricted in their interaction with substrate hydroxyl groups due to steric hindrance.[10] Therefore, lipases prefer the primary carboxylic acids 3-PPA and 4-PBA as substrates. Furthermore, lipases generally exhibit low activity for the esterification of amino acids.[11] This explains why Phe and Trp were not transported through the SLM. Our results suggest that the SLM encapsulating the surfactant–lipase complex may facilitate the selective separation of organic compounds based on the substrate specificity of lipase.

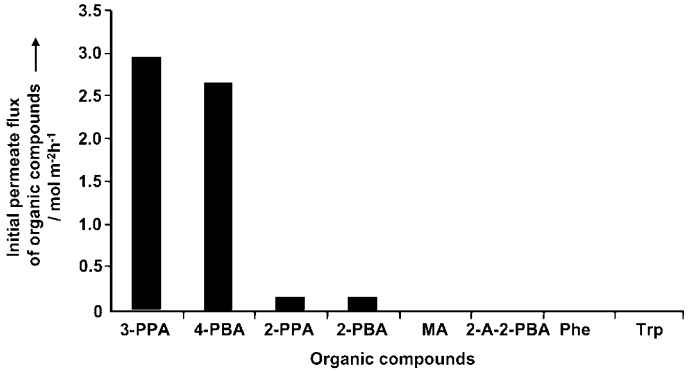

Figure 5. Initial permeate flux of various organic compounds through the SLM encapsulating the surfactant–lipase complex. 3-PPA = 3-phenoxypropionic acid, 4-PBA = 4-phenoxybutyric acid, 2-PPA = 2-phenylpropionic acid, 2-PBA = 2-phenoxybutyric acid, MA = mandelic acid, 2-A-2-PBA = 2-amino-2-phenyl butyric acid, Phe = phenylalanine, and Trp = tryptophan.

Selective separation of organic acids through the SLM encapsulating the surfactant–lipase complex: Based on the results above, this SLM system was expected to facilitate the selective transport through the SLM of a specific organic acid present within a mixture of organic acids. Figure 6 shows the selective separation of organic acids through the SLM encapsulating the surfactant–lipase complex. Three different organic acids, 3-PPA, 2-PBA, and 2-A-2-PBA (10 mM each) were dissolved in the feed aqueous phase and the transport of each was observed. The rate of transport of 3-PPA through the SLM was high; all of it had been transported to the receiving phase after 12 h. The rate of transport of 2-PBA was much lower than that of 3-PPA; the initial permeate flux of 2-PBA (0.17 mol m^{-2} h^{-1}) was one seventeenth of that recorded for 3-PPA (2.97 mol m^{-2} h^{-1}). No transport of 2-A-2-PBA was observed. The differences in transport behavior of the organic acids can be attributed to the substrate specificity of the lipase.[10] These results demonstrate that the SLM encapsulating the surfactant–CRL complex can mediate the selective separation of organic acids based on the substrate specificity of the complex, which will be discussed later.

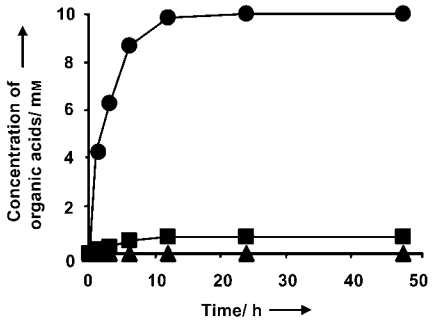

Figure 6. Selective separation of organic acids through the SLM encapsulating the surfactant–lipase complex. Concentration in the receiving phase of 3-PPA (●), 2-PBA (■), and 2-A-2-PBA (▲).

Effect of solvents in the SLM on the permeate flux of organic acids: The solvent used in an SLM is critical for the stability of the liquid membrane phase.[3b,7,12] We employed seven different organic solvents (*n*-hexane, *n*-dodecane, cyclohexane, *n*-pentane, *n*-octane, and *n*-heptane, in addition to isooctane) as the liquid membrane phase (Figure 7a). For all organic acids, the use of isooctane resulted in higher permeate fluxes than those measured with the other solvents. Such differences may have two causes: Firstly, the esterification activity of the surfactant–lipase complex at the liquid–liquid interface or within the organic solvent. It is well known that lipase activity at a liquid–liquid interface and the surfactant–lipase complex activity in an organic solvent are significantly influenced by solvent polarity.[13] Secondly, the solubility of each esterified organic acid in the solvent. Afonso and co-workers also reported the selective separation of organic compounds through an SLM by using ionic liquids, in which the solubility of each compound in the ionic liquid governed the permeate selectivity.[12]

What governs the selectivity in the present SLM?: We examined the esterification activity of the surfactant–CRL complex in the organic solvent–water biphasic systems by using three organic acids as substrates (Figure 7b). The biphasic system consisted of an aqueous buffer containing ethanol and an organic acid, and an organic solvent containing the surfactant–CRL complex (see Experimental Section for details). The isooctane–water biphasic system provided the

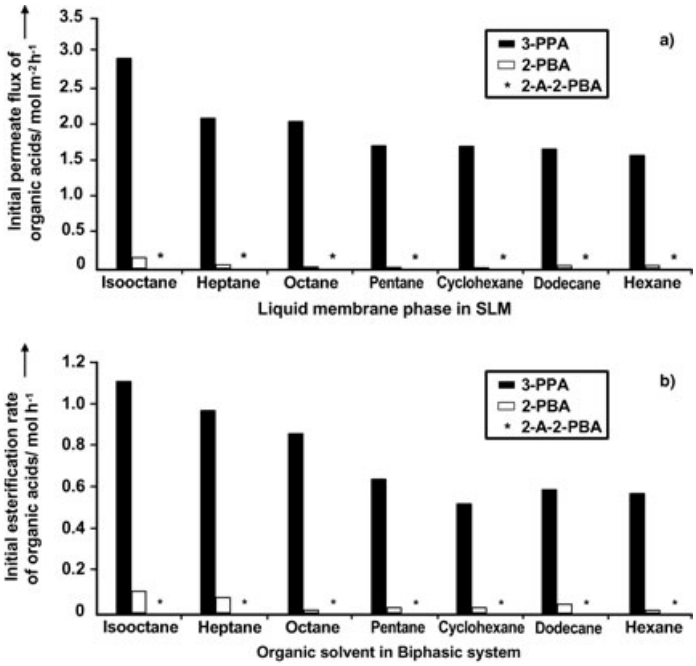

Figure 7. a) Effect of organic solvents in the SLM on the initial permeate flux of organic acids. No transport of 2-A-2-PBA through the SLM was observed (*). b) Effect of organic solvents in the biphasic system on the lipase-catalyzed esterification of organic acids. No esterification of 2-A-2-PBA was observed (*).

highest esterification activity of the hydrocarbon–water systems investigated. In each system, the esterification activity for 3-PPA was notably higher than for the other organic acids. The esterification activity for 2-PBA was much lower than that for 3-PPA, and no esterification of 2-A-2-PBA was observed in any of the systems. These results were consistent with those obtained for the SLM systems.

Furthermore, partitioning coefficients of organic acids and their ethyl esters in the isooctane–water biphasic system were investigated (Table 1). The partition coefficient (P) was defined as the ratio of the concentration of an organic acid (or ethyl ester) in the isooctane phase to that in the aqueous phase. The partition coefficients of 3-PPA and 2-PBA were 0.018 and 0.0050, respectively. The value for 2-A-2-PBA was not determined because it was not detected in the isooctane phase. This explains why the organic acids were not transported through the SLM in the absence of lipases. In contrast, with the exception of 2-A-2-PBA, the partitioning coefficients of the ethyl esters were much higher than those of the free organic acids. In our present SLM system, the ethyl esterification of organic acids promotes the partitioning of the acids into the liquid membrane phase, and the hydrolysis of the ester compound induces the partitioning of the acids into the receiving aqueous phase. To undergo esterification, the organic acids must reach the active site of the surfactant–CRL complex in the liquid membrane. Since 2-A-2-PBA was totally insoluble in the organic phase, it could not reach the lipase and could not be esterified. Our previous study also demonstrated that 2-A-2-PBA could not be esterified by lipase CRL in an aqueous solution. This accounts for the lack of permeation of 2-A-2-PBA through the SLM. On the other hand, the partitioning coefficient of 4-PBA was about 20-fold higher than that of 3-PPA, suggesting good accessibility of 4-PBA to the lipase. The permeate flux of 4-PBA (2.67 mol $m^{-2} h^{-1}$) was, however, slightly lower than that of 3-PPA (2.97 mol $m^{-2} h^{-1}$); this means that the esterification activity of the surfactant–CRL complex for 4-PBA was lower than that for 3-PPA. (Note that the time course of 4-PBA permeation is not shown in Figure 6.) These investigations suggest that the selectivity of the SLM depends on the solubility of the organic acid in the organic phase and on the substrate specificity of the surfactant–CRL complex. The esterification and hydrolysis of 3-PPA, 4-PBA, and 2-PBA were responsible for their permeation through the liquid membrane.

Table 1. Partitioning coefficients of organic acids and their ethyl esters in an isooctane–water biphasic system.

	Partitioning coefficient (P)	
	free organic acid	ethyl ester of organic acid
4-PBA	0.347	0.92
3-PPA	0.018	0.72
2-PBA	0.005	0.47
2-A-2-PBA	N.D.[a]	N.D.[a]

[a] Not determined because 2-A-2-PBA and its ethyl ester were insoluble in the isooctane phase.

Enantioselective transport of (*S*)-ibuprofen through the SLM encapsulating the surfactant–lipase complex: One of the most difficult challenges in the field of fine chemicals is the production and separation of optically active compounds.[14] Lipases have been studied for their potential as biocatalysts for the enantioselective esterification of (*S*)-ibuprofen. The lipase CRL exhibits a particularly high enantioselectivity in the synthesis of ester compounds.[5] We recently reported that the SLM encapsulating the surfactant–CRL complex permitted the highly enantioselective transport of (*S*)-ibuprofen.[6] The enantiomeric excess value ($ee_{48\,h}$ = 91 %) and enantiomeric ratio (E_{value} = 22) obtained for the (*S*)-ibuprofen transported into the receiving phase were greater than those in the previously reported lipase-facilitated SLM system[7] ($ee_{48\,h}$ = 75 %, E_{value} = 3.9). In general, enzymatic reactions in nonaqueous media exhibit different enantioselectivities from those in aqueous solution. Klibanov and co-workers discussed the factors affecting the enantioselectivity in organic solvents with respect to solvation of enzyme and substrates.[15,16]

We investigated the effect of organic solvents in the SLM on the enantioselective transport of (*S*)-ibuprofen. Eight different water-immiscible hydrocarbons were tested as the liquid membrane phase in the SLM system (Figure 8). The enantioselectivity varied from solvent to solvent; for example, the use of isooctane for the liquid membrane phase afforded relatively high enantioselectivity ($ee_{48\,h}$ = 91 %, E_{value} = 22) and the use of *n*-dodecane resulted in fairly low

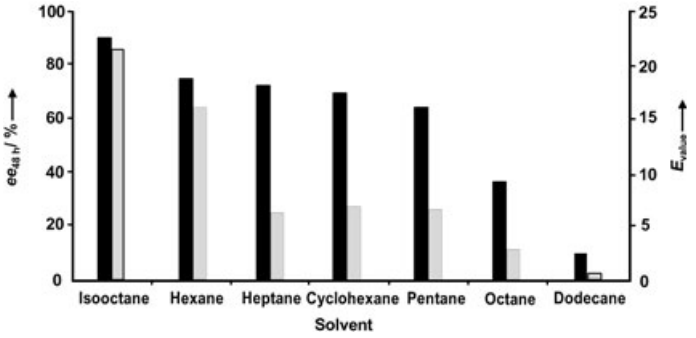

Figure 8. Effect of organic solvents in the SLM on the enantioselective transport of ibuprofen. $ee_{48\,h}=\{([S]_{48\,h}-[R]_{48\,h})/([S]_{48\,h}+[R]_{48\,h})\}\times 100[\%]$, in which $[S]_{48\,h}$ and $[R]_{48\,h}$ are the concentrations of (*S*)- and (*R*)-ibuprofen in the receiving phase after 48 h, respectively (black bars). $E_{value}=J_S/J_R$, in which J_S and J_R are the initial permeate fluxes [mol m^{-2} h^{-1}] of (*S*)- and (*R*)-ibuprofen, respectively (grey bars).

enantioselectivity ($ee_{48\,h}=2.8\,\%$, $E_{value}=1.1$). The high enantioselectivity obtained in the present study could also be due to the organic solvent used in the enzymatic reaction medium.

Effect of the origin of the lipase in the surfactant–lipase complex on the enantioselective transport of (*S*)-ibuprofen: Figure 9a shows the $ee_{48\,h}$ and E_{value} obtained by using lipases of diverse origin in the SLM. Lipase PPL was used as a hydrolysis biocatalyst in the receiving phase. The surfactant–CRL and surfactant–M complexes exhibited high enantioselectivity. The enzyme selected as the esterification biocatalyst was a critical factor in the enantioselective transport of (*S*)-ibuprofen through the SLM.

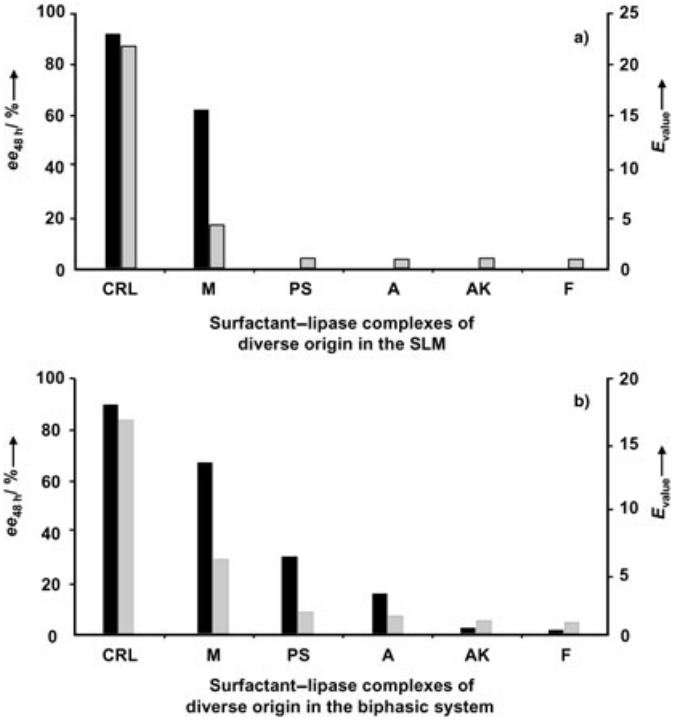

Figure 9. Effect of the origin of lipase used in the surfactant–lipase complex on a) the enantioselective transport of ibuprofen and b) the enantioselective esterification of ibuprofen in the biphasic system. Origin of lipases; CRL (*Candida rugosa*), PPL (porcine pancreas), PS (*Burkholderia cepacia*), A (*Aspergillus niger*), AK (*Pseudomonas fluorescence*), M (*Mucor javanics*), F (*Rhizopus oryzae*). Black and gray bars represent $ee_{48\,h}$ and E_{value}, respectively.

The enantioselectivity of various lipases was also investigated in an isooctane–water biphasic system (Figure 9b). This biphasic system consisted of an aqueous buffer containing ethanol, (*S*)- and (*R*)-ibuprofen, and isooctane containing a surfactant–lipase complex. The enantioselectivity of the lipases in the biphasic system agreed well with that of the SLM system. For example, the surfactant–CRL complex exhibited the highest enantioselectivity in both systems, and the enantioselectivity ($ee_{48\,h}=89\,\%$, $E_{value}=17$) in the biphasic system was very similar to that in the SLM system. These results indicate that the enantioselective lipase enabled the enantioselective separation of organic acids in the SLM.

Conclusion

Our investigations clearly demonstrate that the encapsulation of the surfactant–lipase complex in the SLM facilitates the effective transport and highly selective separation of organic acids through the SLM. The selectivity in the SLM was attributed to the solubility of the organic acid in the organic phase and to the substrate specificity of the surfactant–lipase complex in the liquid membrane phase. By using this SLM, the optical resolution of racemic ibuprofen based on the enantioselectivity of the lipase was possible. This work proves that the combination of nonaqueous enzymology and a conventional SLM technique provides a novel and highly selective separation methodology. However, this SLM system still has several limitations: Not all types of organic compounds can be separated, as the selectivity of this SLM is based on the molecular recognition of lipases. Furthermore, the instability of both the SLM and enzyme activity prevents the continuous operation of the current SLM system. Improvement of stability and the removal of the transported ethanol from the receiving phase could help in achieving a continuous enzyme-facilitated SLM system. We are working on the development of a continuous SLM system encapsulating different biocatalysts.

Experimental Section

General: Lipase CRL and lipase PPL were purchased from Sigma Chemical Corporation, St Louis, USA. Lipases PS, A, AK, M, and F were kindly supplied by Amano Pharmaceutical, Nagoya, Japan. The microporous polypropylene film (Celgard 2500) was obtained from Celgard, Tokyo (Japan). The thickness of the film was 25 μm and the general pore size was 0.2×0.05 μm. 3-PPA, 2-PBA, 4-PBA, Phe, Trp, and ibuprofen were purchased from Wako Pure Chemicals, Osaka (Japan). 2-PPA and 2-A-2-PBA were purchased from Aldrich, Milwaukee (USA). MA was purchased from Tokyo Kasei, Tokyo (Japan). All other reagents were of guaranteed reagent grade. The surfactant dioleyl-L-glutamate ribitol

($2C_{18}\Delta^9$GE) was synthesized according to the procedure described previously.[17]

Synthesis of ethyl esters: The ethyl esters of organic acids were prepared by using the Fisher synthesis.[8b] Typically, organic acid (5 g) and EtOH (300 mL) were dissolved in a flask containing several drops of concentrated H_2SO_4 (as a catalyst), and shaken for 24 h at 60 °C in a fixed reflux condenser. After addition of chloroform (100 mL), the unreacted acid and EtOH were extracted by washing with aqueous $NaHCO_3$. The chloroform phase was dried over anhydrous $MgSO_4$ and filtered, and the solvent was removed by using rotary evaporation. The product was identified by performing thin layer chromatography and ^{1}H NMR spectroscopy.

Preparation of an SLM encapsulating a surfactant–lipase complex: A surfactant–lipase complex was prepared as described previously.[5a] The lipase-immobilized SLM was prepared by immersing a Celgard 2500 film in isooctane containing the surfactant–lipase complex (5 g L^{-1}).

Organic compound transport through the SLM encapsulating the surfactant–lipase complex: Experiments were performed at 37 °C by using a pair of glass cells (cell volume 55 mL, cross-section 5 cm^2, Figure 1b). The SLM separated the two aqueous phases. Typically, the feed phase consisted of ethanol (50 vol %), substrate (10 mM), and McIlvaine buffer (50 vol %, pH 6.3). The receiving phase consisted of McIlvaine buffer (pH 6.3) containing PPL (8 g L^{-1}). Samples (0.5 mL) were periodically withdrawn from the feed and receiving phases and filtered through a 0.5 μm-pore membrane (Millex; Millipore, Billerica, MA). Trichloroacetic acid (0.25 μL, 50 wt % aqueous solution) was added to the filtrates. The mixtures were filtered again and subjected to HPLC analysis. The concentrations of organic acids in the feed and receiving phases were measured by using an HPLC (LC-10 AT; Shimadzu, Kyoto) equipped with an ODS column (4×250 mm; GL Science, Tokyo), eluted with acetonitrile/phosphoric acid (0.1 %) 80:20 (v/v) at a flow rate of 1.0 mL min^{-1}, and with a UV detector at 254 nm.

Regarding the selective permeation of organic acids through the SLM: Each organic acid (3-PPA, 2-PBA, and 2-A-2-PBA; each 10 mM) was dissolved in the feed phase. Transport experiments were carried out as described above. Samples were withdrawn from the feed and receiving phases and subjected to HPLC analysis.

Enantioselective separation of ibuprofen through the SLM encapsulating the surfactant–lipase complex was performed, whereby racemic ibuprofen (10 mM) was dissolved in the feed phase. The concentrations of (*S*)- and (*R*)-ibuprofen in the feed and receiving phases were determined by using an LC-10 AT HPLC equipped with a chiral AD-RH column (4×250 mm; Daicel Chemical Industries Ltd., Tokyo), eluted with acetonitrile/phosphoric acid (0.1 %) 40:60 (v/v) at a flow rate of 0.5 mL min^{-1}, and with a UV detector at 254 nm.

Partitioning of organic acids and ethyl esters in an isooctane–water biphasic system: Partitioning was examined at 37 °C. Each organic acid or ethyl ester (10 mM) was dissolved in McIlvaine buffer (5 mL, pH 6.3) and then added to isooctane (5 mL). After 24 h of incubation, samples (1 mL) were withdrawn from the aqueous and organic phases, filtered through a Millex membrane (pore size 0.5 μm), and subjected to HPLC analysis. The concentration of organic acids and their ethyl esters in the aqueous and the organic phases was measured by using HPLC.

Lipase esterification activity and enantioselectivity in organic solvent–water biphasic systems: Esterification activities were examined at 37 °C. The aqueous phase (0.25 mL) consisted of ethanol (50 vol %), organic acid (10 mM), and McIlvaine buffer (50 vol %, pH 6.3). The organic phase (5 mL) consisted of isooctane containing a surfactant–lipase complex (5 g L^{-1}). Samples (50 μL) were periodically withdrawn from the aqueous and organic phases, filtered through a Millex membrane (pore size 0.5 μm), and subjected to HPLC analysis to determine the concentration of the ethyl esters. The initial rates of ethyl ester production were assumed to represent lipase activity.

Stability of the surfactant–lipase CRL complex in a biphasic system with high ethanol concentration: Stability of the surfactant–lipase CRL complex was measured at 37 °C in a isooctane–water biphasic system. The aqueous phase (1 mL) consisted of ethanol/McIlvaine buffer (pH 6.3) 50:50 (v/v). The organic phase (1 mL) consisted of isooctane containing a surfactant–lipase CRL complex (5 g L^{-1}). After each incubation time (0, 3, 6, 12, 24 h), 3-PPA (10 mM) was added to the solution. Samples (50 μL) were periodically withdrawn from the aqueous and organic phases, filtered through a Millex membrane (pore size 0.5 mm), and subjected to HPLC analysis to determine the concentration of 3-PPA ethyl esters. The initial rates of ethyl ester production were assumed to represent lipase activity.

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