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Lipase−Silicone Biocomposites: Efficient and Versatile Immobilized Biocatalysts

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Abstract: The last few decades have seen an explosion in the application of enzymes to organic chemistry, as these biological catalysts have continued to demonstrate their unique synthetic capabilities. Despite this, a key prerequisite for establishing enzymes as standard reagents in synthetic chemistry, specifically the availability of generic technologies providing inexpensive, robust, and reusable heterogeneous biological catalysts, still remains to be fulfilled. Herein, we describe a novel and highly efficient immobilization methodology for one of the most utilitarian classes of biocatalysts, namely, lipases. The procedure is based upon the adsorption of crude and pure lipases onto poly(hydroxymethylsiloxane), followed by the incorporation of the formed adsorbates into room-temperature vulcanizable silicones, to form biocatalytic composites. This provides hyperactivated catalysts showing activity enhancements of up to 54-fold as compared with the native enzymes, catalytic densities of up to several hundred kilo-units per gram of immobilizate, and high operational activity and stability in aqueous and organic media. The flexibility of silicone polymer chemistry enables the catalytic biocomposites to be prepared with a variety of physicochemistries, and to be fabricated as solid monoliths, sheets of thick films, particulates, and solid foams, thereby allowing the production of tailored catalysts for a variety of applications. The production and properties of a range of lipase−silicone composites are discussed, and the extended performances of selected catalysts are compared with those of the free enzymes and commercial heterogeneous biocatalysts in model synthetic reactions.

Introduction

The utilization of enzymes as versatile and highly selective catalysts is now well established in many areas of synthetic organic chemistry.[1] A central requirement for consolidating biocatalysis as a standard synthetic tool is the development of immobilization methods capable of providing cheap, stable, and efficient heterogeneous biocatalysts. Although newer approaches such as sol−gel encapsulation,[2] cross-linked crystals,[3] and protein−polymer conjugates,[4,5,6] have gone some way toward addressing this issue, generic techniques capable of covering the wide spectrum of synthetically useful enzymes are still lacking. Herein we report a method for preparing highly active, stable, and reusable heterogeneous catalysts from one of the most exploited enzyme classes, namely, lipases,[1,7] based upon the formation of enzyme−silicone polymer composites.

The immobilization of lipases has two important aspects: First, since most lipases appear to show some form of interfacial activation,[8] immobilization should promote this process to the maximum possible extent and prevent the activated enzyme from reverting to the closed form, while minimizing any detrimental conformational changes. Studies have shown that matrices which

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provide both nonpolar/hydrophobic and polar/hydrophilic functionality are critical for achieving optimal lipase activation and stabilization.[9,10] Second, since commercial lipases are often crude preparations with lipase contents as low as 0.1−5% w/w, the immobilization protocol should enable the selective binding of the enzyme from a complex protein mix. Also, lipase immobilization efficiency, activation, and dispersion should be maximized, and protein aggregation minimized,[9,11] at the elevated protein loads required for high catalytic density immobilizates.

Despite the synthetic versatility of lipases and their heavy commercial utilization, comparatively little attention has been directed toward refining immobilization methods. Traditional methods have largely relied upon adsorption onto hydrophobic polymers,[9,11] and only recently have more promising techniques such as sol−gel entrapment and cross-linked crystals been utilized.[2,3] Although polymers such as polypropylene, alkyl-agarose, polyacrylates and polystyrene can selectively bind and activate lipases,[9] enzyme stability can be compromised by hydrophobic polymer surfaces,[11] and enzyme activation and adsorption capacity are typically limited by surface coverage restrictions.[9] Sol−gel entrapment offers mixed functionality matrixes for efficient and stable activation[2l−2q], but is limited to low catalytic densities due to its poor discrimination toward mixtures of proteins in crude lipases, enzyme precipitation in sol−gel solutions at higher protein concentrations, and surface rather than bulk matrix capture of lipase[2l−2q]. Cross-linked lipase crystals can be prepared in a preactivated form, and display excellent activity and stability, but to date this approach has only been applied to two lipases.[3a,−f,i,j]

Results and Discussion

Preparation of *Candida rugosa* Lipase−PHOMS Adsorbates. While studying the encapsulation of proteins in matrices derived from poly(hydroxymethylsiloxane) (PHOMS),[12,13] we discovered that this polymer was a very efficient adsorbent for a variety of hydrophobic molecules, including proteins such as lipases, phospholipases, papain, β-glucosidase, and thermolysin, binding up to 26−34% of its weight of the enzymes from aqueous solutions.[14] Further investigation revealed some unusual properties of PHOMS that made it a promising matrix for immobilizing lipases: (i) PHOMS displays an exceptional capacity for binding lipases, with maximal uptakes being in the range of 28−41% w/w; (ii) PHOMS is available as a micro-dispersed powder (2−13 μm) with a high surface area (451−872 $m^2\ g^{-1}$) and pore volume (0.38−1.23 mL g^{-1}), which undergoes considerable surface area/porous volume expansion

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(10) Investigations on the interactions of lipases with Langmuir−Blodgett Layers (LBLs) and engineered polymers have demonstrated that hydrophobic surfaces can initiate gross conformational disturbances in the adsorbed species, leading to protein unfolding, and ultimately, diminished activity and stability.[11]

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(13) PHOMS can be prepared by oxidizing poly(hydrogenmethylsiloxane) with dimethyl dioxirane (DMDO),[12] or on the large scale more conveniently by transfer dehydrogenation with acetone, using Zn(II)/Sn(II) as catalysts.

(14) The addition of PHOMS (20% w/w of a 50% w/w paste in 4:1 water−propan-2-ol) to aqueous protein solutions (10 mg mL^{-1} in 0.1 or 0.2 M metaphosphate buffer, pH 7, containing 20 mM calcium acetate, 5 °C), at an applied protein load of 10% w/w of PHOMS, gave the following activity immobilizations: papain, 96%; thermolysin, >99%; almond β-D-glucosidase, 98%; *C. rugosa* lipase-B, >99%; *S. chromofuscus* phospholipase D, 98%. Adsorption took 10−30 min, and less than 3% leaching of protein/activity was detected upon washing (3 × 10-fold volumes of buffer, 5 °C, 1 h).

Table 1. Properties of Lipases Used in Immobilization Studies

enzyme	form	protein content (% w/w)[a]	specific activity of enzyme: 4-NPB (μm min^{-1} mg^{-1})[b]	TO (μm min^{-1} mg^{-1})[c]	LA+OcOH non-act[d]	(μm h^{-1} mg^{-1}) activated[e]
A. niger	crude	86 (57)	0.68	1.4	0.78	2.95 (3.8)
C. rugosa	crude	11 (8)	32.3	48	9.22	43.3 (4.7)
C. rugosa	pure	78	5650	8450	711	2340 (3.3)
C. antartica	crude	29 (13)	1.10	3.3	5.31	9.54 (1.8)
M. miehei	crude	9 (8)	2.03	2.1	5.86	15.1 (2.6)
M. javanicus	crude	47 (26)	86	411	73.1	8.06 (2.8)
Porcine pancreas	crude	24 (11)	1.03	1.9	13.5	255 (27.4)
Porcine pancreas	pure	71 (63)	418	906	351	16530 (18.6)
P. cepacia	crude	2.6 (3.1)	0.06	0.13	0.91	6.90 (7.6)
P. cepacia	pure	24 (19)	23.1	57	373	2720 (7.3)
P. fluorescens	crude	14 (11)	19.4	49	106	835 (7.9)
P. roquefortii	crude	23 (15)	1.38	3.2	1.91	5.92 (3.1)
R. arrhizus	crude	19	1.06	6.8	0.59	18.7 (31.7)
R. arrhizus	pure	78	1350	9712	287	2750 (9.6)

[a] The first figure and those in parentheses respectively indicate the protein contents as determined by the Lowry method without and with prior precipitation of the protein by trichloroacetic acid. [b] Hydrolysis of dissolved 4-nitrophenyl butyrate (4-NPB) (5 mM in 50 mM Tris buffer, pH 7.5, 10% v/v propan-2-ol, 30 °C) in aqueous buffer. [c] Hydrolysis of a triolein (TO) (0.15 M in 5 mM Tris buffer pH 8, 25 mM Triton X-100, 30 °C). [d] Non-activated, lyophilized enzyme in the esterification of lauric acid (LA) with octanol (OcOH) (50 mM and 100 mM respectively in buffer-saturated isooctane, 30 °C). [e] Activated, lyophilized enzyme in the esterification of LA with OcOH in isooctane. The activation factor is given in brackets. The activities are given per mg of crude or purified enzyme preparation. The specific activities of the lyophilized materials are given on the original mass basis, prior to freeze-drying, to correct for mass changes due to the inclusion of buffer salts, etc.

when dispersed in aqueous milieu;[15] (iii) PHOMS has a mixed surface coverage of methyl, silanol, and siloxane groups, the relative contents of which can be modified via silylation and pH and thermal treatments;[16] (iv) its silanol functionality enables PHOMS to be used as an active filler in room-temperature vulcanizable (RTV) silicone compositions, thereby furnishing solid composite materials with the typical physicochemical attributes of filled silicone polymers.[17,18]

The suitability of PHOMS as an immobilization matrix was determined by evaluating its performance with *C. rugosa* lipase, an enzyme which has been widely utilized in enantioselective syntheses in its free, immobilized, and cross-linked crystal forms.[1,2m−p,3e,f,i,j,7] First, the ability of PHOMS to bind *C. rugosa* lipase from purified and crude enzyme preparations (Table 1) and the hydrolytic activities of the adsorbates toward 4-nitrophenyl butyrate (4-NPB) were examined (Figure 1). The adsorption capacity of PHOMS plateaued at ∼300−320 mg of lipase protein per gram of polymer, with greater than 90% immobilization of lipase activity from solution being observed up to protein−PHOMS ratios of 150−175 mg per gram of adsorbent. The similar adsorption profiles obtained for the crude and purified enzyme testify to the ability of PHOMS to selectively bind the lipase from complex protein mixtures.[19] These results compare very well with described adsorption and sol−gel encapsulation routes which are limited to lipase loads of 1−6% w/w and 0.5−2% w/w respectively due to surface saturation and protein precipitation/aggregation effects at higher dopings.[3a−e,9] It was also found that sol−gel methyl-, ethyl- and propyl-siloxanes were much inferior adsorbents to PHOMS, exhibiting maximal uptakes of 13−17% w/w and specific activity declines above 3−5% w/w loading. It is conceivable that the open structure, mixed display of hydrophilic silanol and hydrophobic methyl functionality, and high porosity and surface area of PHOMS may provide a unique environment for lipase attachment.[11]

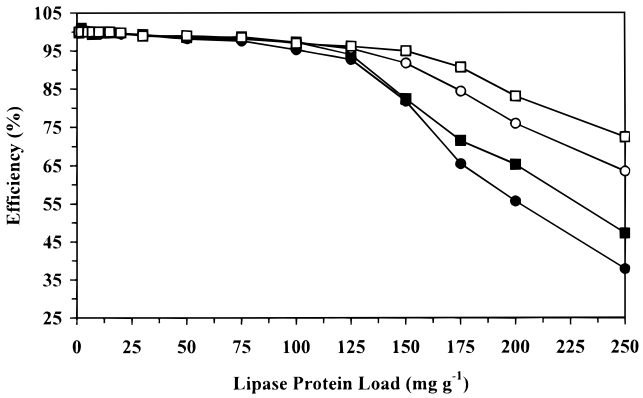

Figure 1. Effect of loading on immobilization efficiency and relative specific activity of *C. rugosa* lipase−PHOMS adsorbates. Adsorption (%) of total lipase activity from solution of purified (□) and crude (○) enzyme, and relative specific activity (4-nitrophenyl butyrate assay) of purified (■) and crude (●) lipase−PHOMS adsorbates. The highest specific activities of the crude and purified enzyme−PHOMS immobilizates (100% in graph) were 2.44 and 2.73, respectively, relative to the free enzymes. PHOMS with a silanol content of 7.2 mmol g^{-1}, pore volume of 2.63 mL g^{-1}, and surface area of 1408 m^2 g^{-1} was used.

(15) The figures for dry PHOMS are given. Samples were micro- to meso-porous, with upper pore size limits of 6−9 nm. Immersion in aqueous and aqueous−organic media and polar organic solvents lead to swelling of the particulate structures by ∼60−320%. Thermoporometric analysis (TPA) of PHOMS hydrated in pure water indicated considerable expansion of the porous volume and surface area, with upper pore distributions ranging over 8−21 nm, pore volumes of 1.42−3.07 mL g^{-1}, and surface areas of 672−1643 m^2 g^{-1}.

(16) The silanol content of PHOMS varied between 6 and 12.3 mmol/g (46−93% of theoretical maximum), depending upon the mode of preparation and the aging and drying conditions. Wet processing at low pH and/or high temperature, and/or dry calcination affords a low-silanol/high-siloxane PHOMS. Conversely, low temperature and/or high pH treatment afford highly silanolated products. Silanol coverage was determined by titration with LAH, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).

(17) The silanol groups of PHOMS react with the alkoxysilane functionalities of poly(alkyl silicate) to form siloxane bridges.[18] This condensation is catalyzed by Sn(II), Fe(III), Ti(IV), Zn(II), Pt(IV), and other metal salts.

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(19) It was calculated that *C. rugosa* lipase constituted less than 1% w/w of the total protein content of the crude enzyme preparation. Crude *C. rugosa* enzyme contains a number of lipase components. The adsorption of total lipolytic activity was followed, and no attempt was made to profile the adsorption of the various lipase species.

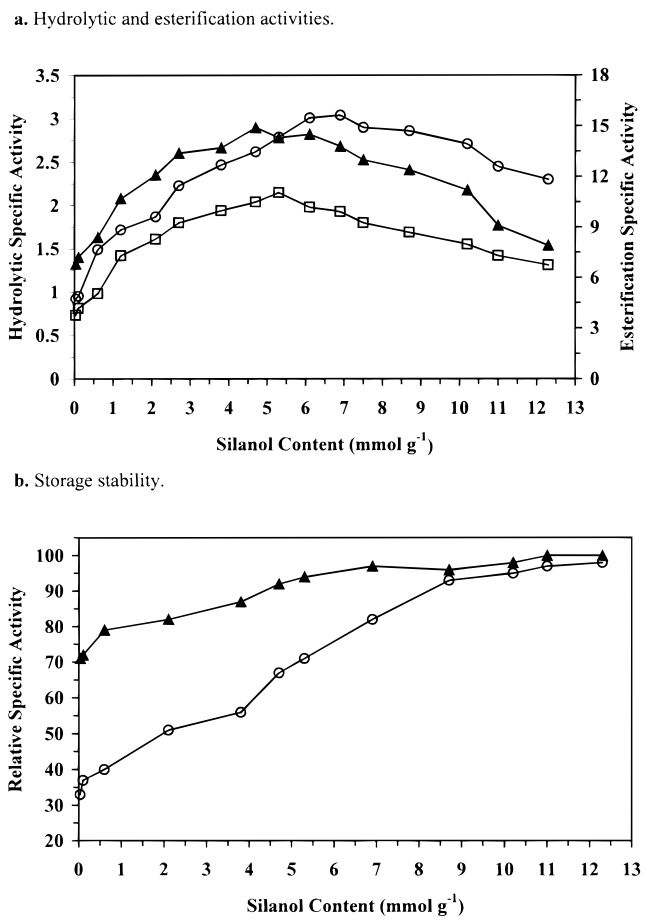


Figure 2. Effect of silanol content of PHOMS on lipase activity and stability of *C. rugosa* lipase B. Dependence of specific activity (a) and storage stability (b) of *C. rugosa* lipase B−PHOMS adsorbates upon the silanol content of PHOMS. (a) ○: hydrolysis of 4-nitrophenyl butyrate (5 mM in 50 mM Tris buffer, pH 7.5, 10% v/v propan-2-ol, 30 °C); □: hydrolysis of triolein (0.15 *M* in 5 mM Tris buffer pH 8, 25 m*M* Triton X-100, 30 °C); ▲: esterification of lauric acid with octanol (50 m*M* and 100 m*M*, respectively, in buffer-saturated isooctane, 30 °C). (b) ○: storage in phosphate buffer (0.1 M, pH 7.5, containing 25 mM calcium acetate); ▲: storage in isooctane saturated with buffer. PHOMS with a silanol content of 7.2 mmol g^{-1}, pore volume of 2.63 mL g^{-1} and surface area of 1408 m^2 g^{-1} was used for silylation/adsorbate preparation. The adsorbates were stored at room temperature for six months, and the activity losses determined from 4-NPB hydrolysis.

The influence of PHOMS surface functionality on *C. rugosa* lipase B immobilizates was then examined using PHOMS whose silanol sites were capped to varying extents by trimethylsilylation (Figure 2). Capping little affected the surface area or porosity of PHOMS,[20] and did not greatly alter the capacity (28−31% w/w at saturation) or immobilization efficiency (above 99% at 10 mg g^{-1} loading). However, hydrolytic activity toward soluble 4-NPB and triolein (TO) emulsions and esterification activity toward lauric acid (LA)/octan-1-ol (OcOH) in isooctane peaked at silanol contents ranging over 4−8 mmol g^{-1} (Figure 2a), whereas catalyst stability upon storage in buffer and isooctane increased steadily with silanol coverage (Figure 2b). This accords with studies on the influence of support surface chemistry in determining lipase activity and stability.[11]

Next, we examined the catalytic performances of the *C. rugosa* lipase B−PHOMS immobilizates (Figure 2a). The specific activities for the hydrolysis of 4-NPB and TO were higher than those of the free enzyme (at silanol coverages above 0.6 mmol g^{-1}), climaxing at 3.01 ± 0.18 and 2.15 ± 0.15, respectively, and in esterification an enhancement of up to 14.9 ± 0.8-fold was observed. Since the use of the purified lipase at a low load (10 mg g^{-1}) should have minimized aggregation effects and the influence of contaminating proteins, it is tempting to ascribe these results to PHOMS-induced conformational changes, including lid mechanism activation, although altered substrate/product partitioning could also be invoked. Interestingly, preactivation of *C. rugosa* lipase B by lyophilization in the presence of octyl β-D-glucopyranoside,[21] produced a rate increase of 3.3 ± 0.14-fold over the untreated enzyme in the esterification reaction. The further enhancement of some 4.5 ± 0.21-fold for the PHOMS adsorbate may suggest the operation of distinct support-lipase interactions, perhaps most importantly lipase dispersion, in addition to lid displacement and open form stabilization.

Production of *C. rugosa* Lipase−PHOMS−Silicone Composites. Although the lipase−PHOMS adsorbates were highly active, they were not suitable heterogeneous biocatalysts due to their fine dispersion, which made reuse difficult, and because of possible desorption of the enzyme upon long-term use. Thus, for recycling purposes, it was necessary to recover the adsorbates by centrifugation. Also, studies with PHOMS adsorbates of *C. rugosa*, *Pseudomonas cepacia*, *P. fluorescens*, *Mucor miehei*, and porcine pancreatic lipases indicated that 6−16% of lipase activity was desorbed upon twelve cycles of washing with buffer containing 5% v/v 2-propanol.[22]

Therefore, the second part of the immobilization protocol entailed the formation of solid lipase−PHOMS−silicone polymer composites by combining the adsorbates (20−30% w/w) with a mixture (70−80% w/w) of silanol-terminated poly(dimethylsiloxane) prepolymers, poly(diethylsilicate) and poly(3-aminopropylethoxysilane) cross-linkers, and tin(II) 2-ethylhexanoate catalyst (Scheme 1).[17] Medium- and long-chain (22−340 DPs) prepolymers and a linear cross-linker were utilized so as to obtain a low cross-link density and secure flexible and open polymer frameworks. This protocol provided solid silicone rubbers with encapsulated lipase−PHOMS, which could be cast/processed to furnish biocatalysts in the form of monoliths, granules, powders, thin sheets, and coatings (Chart 1). Almost quantitative encapsulation (97−100%) was observed, and importantly, the composites obtained from both crude *C. rugosa* lipase and purified *C. rugosa* lipase B appeared to substantially retain the high enzyme activations observed for the lipase−PHOMS adsorbates (Figures 3 and 4). Thus, the hydrolytic activities toward 4-NPB and TO were 1.83−1.91 and 1.75−1.92, respectively, and the esterification activity toward LA was in the range of 10.7−12.2 when compared with the nonactivated lipases, and 2.3−3.6 when referred to the activated enzymes.

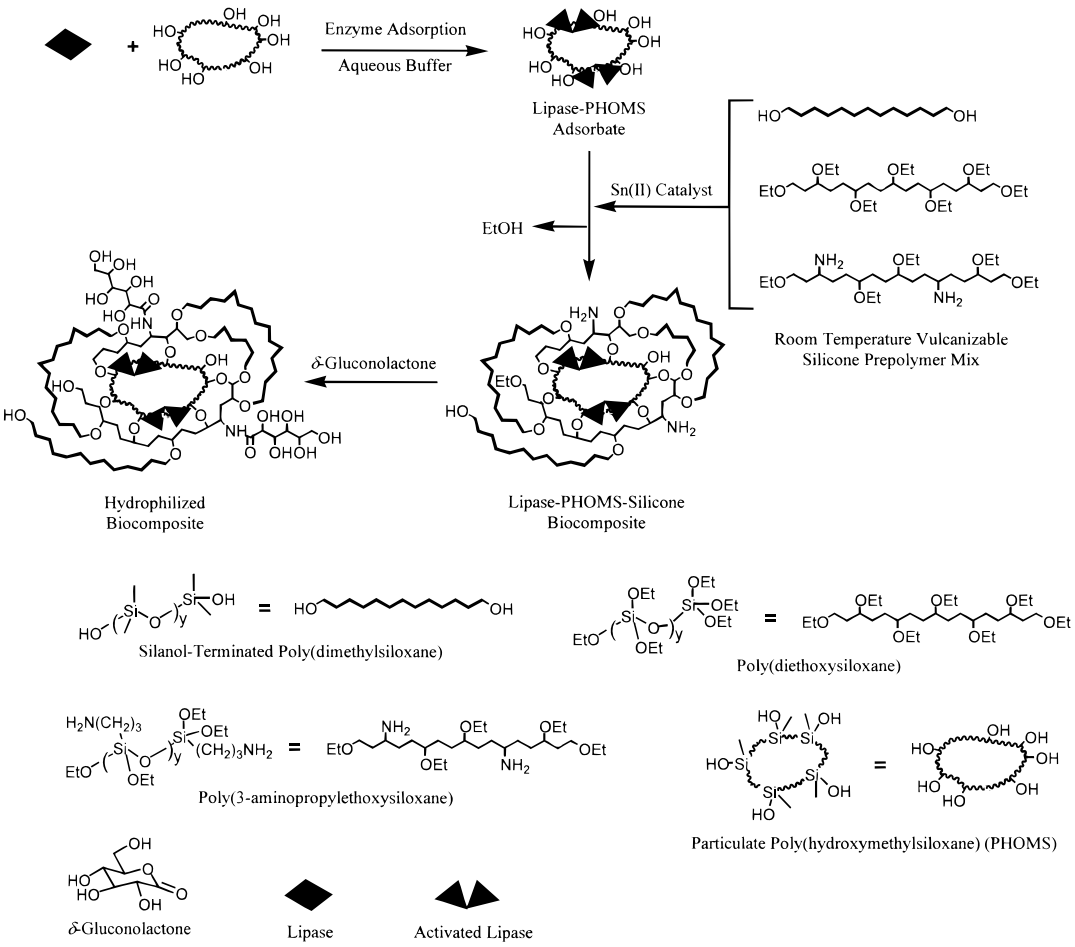
General Application of PHOMS−Silicone Encapsulation Technique to Lipases. The general applicability of the immobilization technique was evaluated using a range of synthetically useful lipases (Tables 1 and 2). PHOMS with a silanol

(20) The surface area and pore volume of the PHOMS used in the study were 813 m^2 g^{-1} and 1.17 mL g^{-1} respectively. Trimethylsilylation reduced these to 746−781 m^2 g^{-1} and 0.94−1.02 mL g^{-1}.

(21) (a) Mingarro, I.; González-Navarro, H.; Braco, L. *Biochemistry* **1996**, *35*, 9935. (b) Mingarro, I.; Abad, C.; Braco, L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3308.

(22) Lipase−PHOMS adsorbates of *C. rugosa*, *Pseudomonas cepacia*, *P. fluorescens*, *Mucor miehei*, and porcine pancreatic lipases (0.5−1 g) prepared with lipase protein loadings of approximately 50 mg g^{-1} were exposed to 12 cycles of washing (15−30 mL of 50 mM phosphate, pH 7, containing 5% v/v 2-propanol, 15−20 h). 4-NPB hydrolysis and Lowry assays indicated that 6, 12, 13, 9, and 16% of lipase activity was desorbed respectively and that the adsorbates retained 91, 80, 83, 87, and 77% of their initial activities.

Scheme 1. Formation of Lipase−PHOMS−Silicone Composite Biocatalysts



content of 9.3−10.1 mmol g^{-1} was selected, and a lipase protein load of 20 mg g^{-1} (5.4−31 kU g^{-1}, triolein (TO) hydrolysis activity) was used in the studies. Both crude and purified enzymes were sequestered with activity immobilizations of 94−99% (Table 2), which, considering the low lipase contents of the crude preparations, attest to the selectivity of PHOMS for binding lipases. Thus, 1 g of PHOMS bound 94−95% of the lipolytic activity (∼19−21 mg of pure lipase) from the aqueous extracts obtained from over 30 g of crude *P. cepacia* and *Rhizopus arrhizus* enzymes. In these preparations the lipase components amounted to less than 1% w/w of the total protein contents. Also, this efficiency was maintained for binding up to ∼150 mg of lipase protein per gram of polymer. Similar selectivities, albeit at much lower loads, have been reported for octyl-agarose, polypropylene and polyacrylates.[9] It should be noted that in many cases lipids, cell debris, carbohydrates, and salts rather than soluble proteins may be the major contaminants of crude lipase preparations, and in these instances lipase binding selectivity may not be such an issue.

The lipase−PHOMS adsorbates proved to be efficient catalysts (Figures 3 and 4), showing activity enhancements of 1.4−8.22- and 1.16−3.1-fold over the free enzymes in the hydrolysis of 4-NPB and TO respectively (Figures 3a and 3b). Also, esterification rate increases of 4.6−48.1 were seen for the reaction of LA with OcOH (Figure 4a), mirroring those described for sol−gel entrapped lipases.[3a−3d] Interestingly, when compared with the preactivated lipases, the esterification activities of the adsorbates ranged over 1.8−7.7 (Figure 4b). Thus, activation could account for a large proportion of the observed performance increases, and the inferior profiles of the native enzymes in nonaqueous media may be attributable to the absence of enzyme activation,[23] combined with protein aggregation and poor protein dispersion.

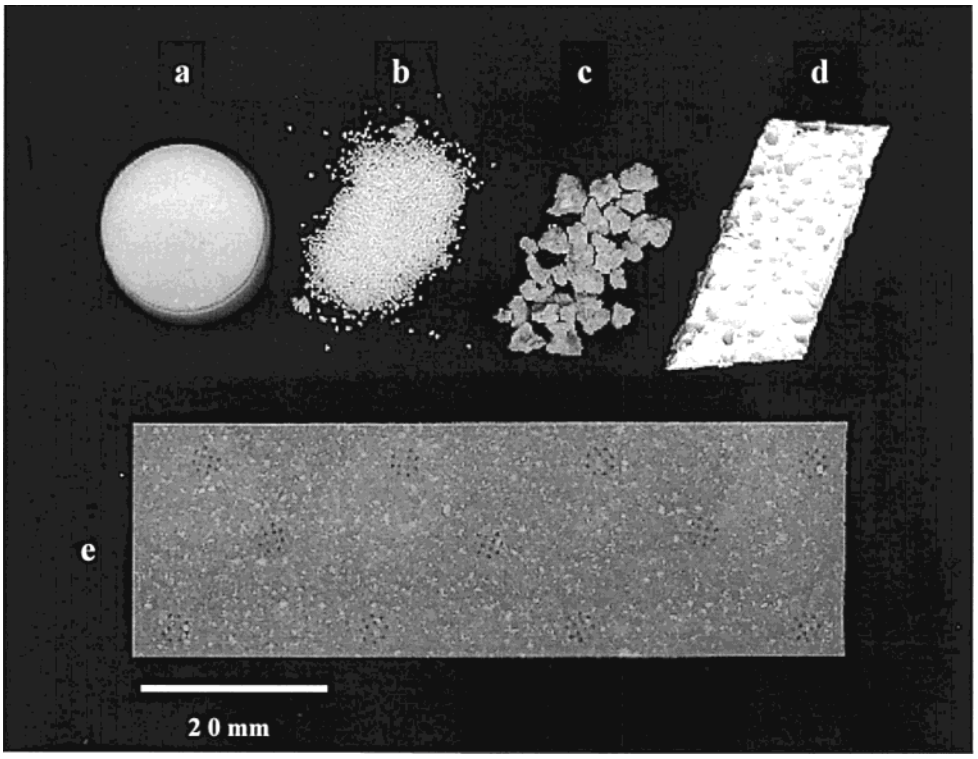
Silicone composites encapsulating 25% w/w of the lipase−PHOMS adsorbates were fabricated as described above, and their performances examined.[24] The hydrolytic activities toward 4-NPB and TO were 1.13−6.35 and 0.93−2.72 times higher, respectively, than those of the free enzymes (Figure 3), and esterification rate increases of 4.1−53.6-fold over those of the nonactivated enzymes, and 1.6−7 times greater than those of the activated lipases, were obtained (Figure 4).

It is important to note that the versatility of silicone polymer chemistry,[18] can be utilized to tailor the properties of the biocatalytic composites to suit the enzyme, fabrication requirements, and final applications. Thus, depending upon the prepolymer mix and lipase−PHOMS content (5−40% w/w), physicochemically robust materials ranging from flexible, translucent rubbers to rigid, opaque solids can be produced as bulk solids, sheets, films, and particulates that are stable in aqueous and organic media and suited to long-term catalyst usages. The inclusion of poly(hydrogenmethylsiloxane) as a blowing agent furnishes solid macroporous foams (Chart 1) which are fitting for use with gaseous substrates. Also, PHOMS as well as the composites can be modified through silylation; thus, treatment of PHOMS with alkylsiloxanes prior to binding

(23) Louwrier, A.; Drtina, G. J.; Klibanov, A. M. *Biotechnol. Bioeng.* **1996**, *50*, 1.

(24) Titration with lithium aluminum hydride indicated that the silanol functionality available for cross-linking was reduced by 13−21% upon protein adsorption.

Chart 1. Representative Examples of Lipase−PHOMS−Silicone Composite Biocatalysts[a]



[a] Top left to right: (a) solid monolith, (b) 100−300 μm granulate, (c) thick film composite coated onto a granular clay support, (d) macroporous foam composite blown with PMHS, (e) 1 mm composite sheet.

of *C. rugosa* lipase B furnished more hydrophobic adsorbents which led to lipase−PHOMS adsorbates and lipase−silicone composites with increased hydrolytic and synthetic activities (Figure 3, and Table 2, Entries 4 and 5). Alternatively, the composites can be functionalized using modified siloxane prepolymers, for example, incorporation of poly(3-aminopropylethoxysiloxane) or poly(3-glycidoxypropylethoxysiloxane) cross-linkers into the silicone mixes, followed by treatment with δ-gluconolactone/pantolactone or ethanolamine/glucosamine respectively provides hydrophilized composites which are better suited to aqueous and polar organic media. Indeed, a gluconamidated *C. rugosa* lipase B-silicone composite provided significantly better performance in aqueous and organic media (Figure 3, and Table 2, Entry 6).

Next, we examined the critical question of the storage and operational stabilities of the lipase−silicone biocatalysts (Table 2). Dry and wet storage of the composites at room temperature over six months resulted in the respective loss of only 0−4% and 2−15% of the initial activities. Also, when the catalysts were reused over 480 h for TO hydrolysis and LA esterification, they held 71−92% and 83−95% of their starting activities respectively, demonstrating their working stability in aqueous and low-water media. In comparison, the lipase−PHOMS adorbates retained 42−62% and 49−77% of their initial activities under the same operational conditions (Table 2), the lower figures probably reflecting a combination of enzyme desorption and surface inactivation. The results also indicate that lipase activation is not a transient phenomenon but is stably retained in the composites upon prolonged storage or use.

Synthetic Applications of Lipase−PHOMS−Silicone Composites. Having established that highly active and stable lipase−silicone composite polymers could be produced, we examined their synthetic utility in four model enzymatic syntheses (Table 3). Native, cross-linked crystalline and silicone composite-encapsulated *C. rugosa* lipase B were used for the enantioselective hydrolysis of (*R,S*)-ibuprofen methyl ester (**1**) in an aqueous−organic system to produce enriched (*S*)-ibuprofen (**2**).[25d] The cross-linked crystalline and composite catalysts displayed initial enantioselectivities of over 100, and even after 10 cycles of use these were retained at 35 and 41%, respectively, of their initial values. The specific activities of the immobilizates were approximately 4-fold higher than the free enzyme and were well maintained, ending at 88 and 90%, respectively, of the initial values. In contrast, the selectivity of the free enzyme was much lower, and its reuse in the aqueous−organic reaction medium proved impractical due to difficulty in recovering the enzyme.

The reverse procedure, namely enantioselective esterification with methanol in dichloromethane was utilized for the *C. antartica*-mediated resolution of (*R,S*)-suprofen (**3**) into the desired, unreacted (*S*)-suprofen (**4**) and (*R*)-suprofen methyl ester.[25a] In this case the enantioselectivities of the free and immobilized catalysts were very similar, and virtually unaffected after 10 cycles of catalyst reuse. However, the initial activity of the composite catalyst was 3 to 4 times higher than the that of the free enzyme or of Novozym 435. In addition, the activity of the free enzyme dropped by 70%, whereas those of the commercial immobilizate and composite catalysts lost only 19 and 3% of their activities, respectively.

A regioselective transformation, the aminolysis of the dibenzyl ester (**5**) to afford the monophenylethylamide (**6**),[25b] was used to assess the performances of *P. cepacia* catalysts. The composite catalyst proved to be considerably more active than the native lipase preparations and displayed a high operational stability, retaining 95% of its activity compared with 38 and 84% for the free enzymes.

(25) (a) Mertoli, P.; Nicolosi, G.; Patti, A.; Piatelli, M. *Chirality* **1996**, *8*, 377. (b) Adamczyk, M.; Grote, J. *Tetrahedron Lett.* **1996**, *37*, 7913. (c) Allen, J. V.; Williams, J. M. J. *Tetrahedron Lett.* **1996**, *37*, 1859.

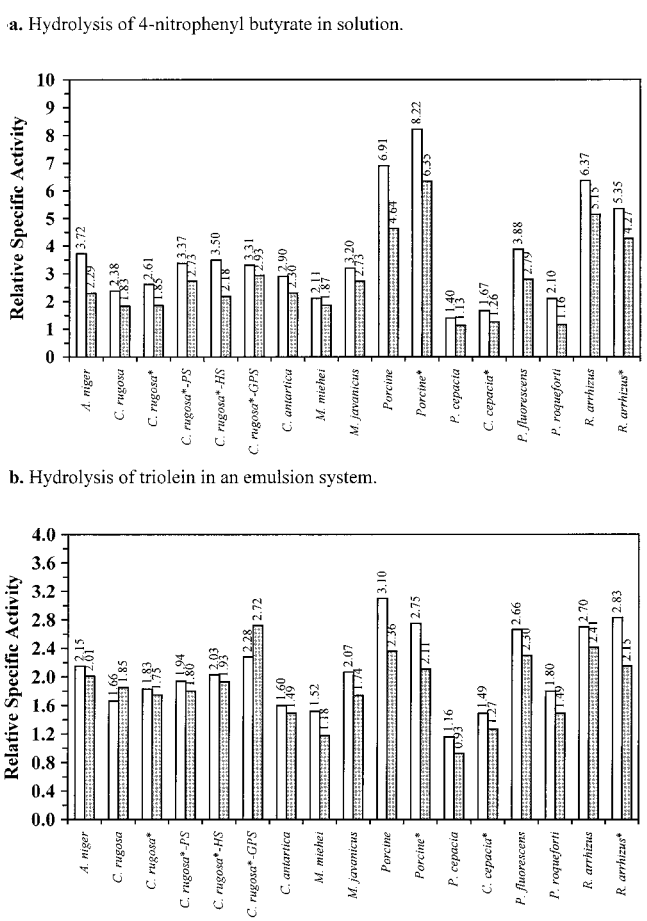


Figure 3. Hydrolytic activities of lipase−PHOMS and lipase−silicone biocatalysts. The relative specific activities in hydrolysis of the lipase−PHOMS adsorbates (unfilled bars), and the lipase−PHOMS−silicone composites (filled bars) are shown. (a) hydrolysis of 4-nitrophenylbutyrate (5 mM in 50 mM Tris buffer, pH 7.5, 10% v/v propan-2-ol, 30 °C); (b) hydrolysis of triolein (0.15 M in 5 mM Tris buffer pH 8, 25 mM Triton X-100, 30 °C). All activities are compared against those of the corresponding free enzymes in solution (specific activity 1.0). Adsorbates were prepared using PHOMS with a silanol content of 9.3−10.1 mmol g^{-1}, pore volume of 2.41−2.97 mL g^{-1} and surface area of 1502−1570 m^2 g^{-1}, and an applied lipase load of 5 mg g^{-1} of PHOMS. Composites were fabricated using 25% w/w of adsorbate, 56% w/w of PDMS prepolymers, and 19% w/w of cross-linker (4:1 PDES−PAPES), hydrophilized with δ-gluconolactone, and then granulated, and the 100−300 μm fractions were used. Asterisks indicate the purified enzymes. Asterisks indicate the purified lipases. Abbreviations: *C. rugosa**−PS, prepared from propylsiloxylated-PHOMS; *C. rugosa**−HS, prepared from hexylsiloxylated-PHOMS; *C. rugosa**−GPS, prepared from 3-gluconamidopropylsiloxylated-PHOMS.

Finally, we examined a dynamic reaction using a lipase-transition metal complex combination catalyst, namely the *P. fluorescens*/Pd(II)-mediated resolution of the cyclohexenyl acetate (**7**) to give the (−)-cyclohexenol (**8**).[25c] In this protocol, the lipase mediates the enantioselective hydrolysis of the racemate to give enriched (−)-alcohol, while the bis(acetonitrilato)palladium(II) chloride complex simultaneously racemizes the starting acetate while leaving the product alcohol unchanged. The initial activity of the biocomposite was over 5-fold that of the commercial immobilizate, and this fell by only 10% with recycling in contrast to the 45% loss observed for the latter catalyst. Furthermore, a much higher enantiselectivity was observed for the silicone biocatalyst, and this was substantially retained after reuse.

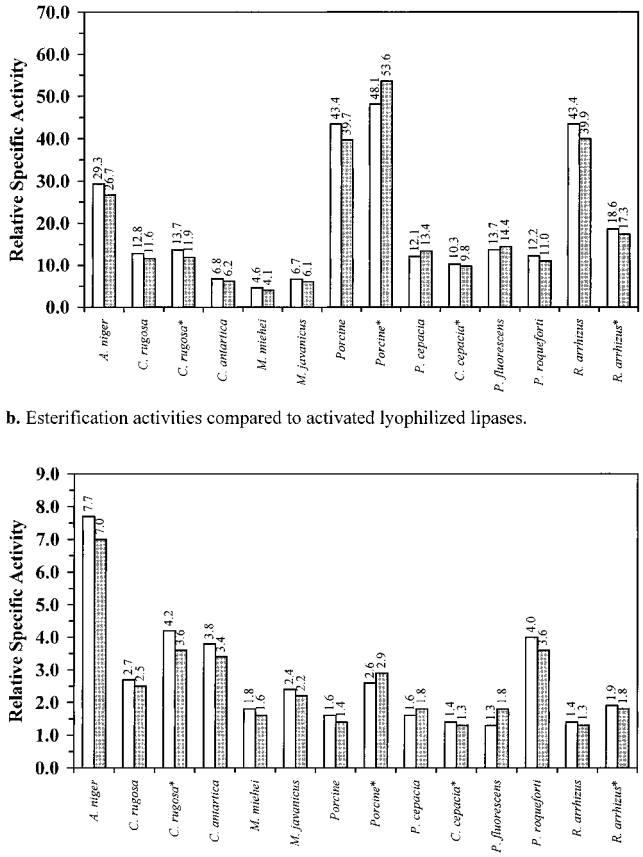


Figure 4. Esterification activities of lipase−PHOMS and lipase−silicone biocatalysts. The relative specific activities of the lipase−PHOMS adsorbates (unfilled bars), and the lipase−PHOMS−silicone composites (filled bars, 25% w/w of lipase−PHOMS in composite) in the esterification of lauric acid with octanol (50 mM and 100 mM, respectively, in buffer-saturated isooctane, 30 °C) are given. (a) Compared against lyophilized nonactivated enzymes; (b) compared against lyophilized activated lipases. Adsorbates were prepared using PHOMS with a silanol content of 9.3−10.1 mmol g^{-1}, pore volume of 2.41−2.97 mL g^{-1}, and surface area of 1502−1570 m^2 g^{-1}, and an applied lipase load of 5 mg g^{-1} of PHOMS. Composites were fabricated using 25% w/w of adsorbate, 56% w/w of PDMS prepolymers, and 19% w/w of cross-linker (4:1 PDES−PAPES), hydrophilized with δ-gluconolactone, and then granulated, and the 100−300 μm fractions were used. Asterisks indicate the purified enzymes.

Conclusions

The PHOMS−silicone encapsulation technique offers an efficient route for generating immobilized lipase catalysts. Catalytic densities of a few units to hundreds of kilo-units per gram of biocomposite can be readily obtained, and this together with the flexibility to engineer the polymer support enables one to tailor the biocatalyst to the final application. The approach appears to satisfy the main requisites for producing efficient immobilized lipase catalysts, namely the effective binding of large amounts of protein, the stable hyperactivation of the enzyme, and the exhibition of high activity and stability in different media. Considering the facile and low-cost chemistries required for producing these biocomposites, and their amenability to mass production,[26] these catalysts should prove practical for industrial applications and should usefully complement sol−gel and cross-linked crystal biocatalysts. In addition, initial trials with proteases, glycosidases, oxidoreductases, and aldolases, bound to PHOMS or to functional silane-modified PHOMS have

Table 2. Properties and Storage and Operational Stabilities of Lipase−PHOMS−Silicone Composite Biocatalysts

lipase preparation	form	lipase load applied[a] kU	lipase load applied[a] mass	% lipase activity immobilized[b] PHOMS	% lipase activity immobilized[b] LSC	storage stability (6 months)[c] dry	storage stability (6 months)[c] wet	working stability TO hydrolysis PHOMS	working stability TO hydrolysis LSC	(over 20 cycles)[d] esterification PHOMS	(over 20 cycles)[d] esterification LSC
A. niger	crude	2.72	4.00 g	96	95	98	93	-	81	-	87
C. rugosa	crude	144	4.48 g	98	96	97	96	49	86	69	88
C. rugosa	pure	144	25.6 mg	99	99	100	98	53	88	72	91
C. rugosa[e]	pure	144	25.6 mg	99	99	98	95	60	92	71	93
C. rugosa[f]	pure	144	25.6 mg	98	96	-	-	62	-	74	90
C. rugosa[g]	pure	144	25.6 mg	99	98	-	-	55	83	77	91
C. antartica	crude	1.76	1.60 g	99	98	100	89	-	86	-	86
M. miehei	crude	1.64	0.80 g	96	94	99	97	59	80	68	84
M. javanicus	crude	1.72	0.20 g	97	97	97	86	-	85	-	92
Porcine pancreas	crude	11.6	11.28 g	97	95	96	85	47	90	56	94
Porcine pancreas	pure	11.6	27.6 mg	98	98	99	91	-	-	-	-
P. cepacia	crude	2.0	33.3 g	93	94	98	91	-	71	-	83
P. cepacia	pure	2.9	86.8 mg	99	97	98	95	42	78	49	85
P. fluorescens	crude	4.0	0.210 g	99	99	99	86	47	83	52	89
P.roqueforti	crude	0.8	0.616 g	95	92	97	91	-	89	-	93
R. arrhizus	crude	34	32.1 g	95	92	96	86	-	85	-	94
R. arrhizus	pure	34	25.1 mg	99	98	-	-	-	88	-	95

[a] Amount of lipase used per gram of PHOMS, as 4-NPB hydrolysis units and actual mass. [b] Overall percentage of initially applied lipase activity in solution that was immobilized in lipase−PHOMS adsorbate (applied lipase load of approx. 20 mg g^{-1}) and final silicone composite (LSC, lipase−PHOMS content of 25% w/w). [c] Percentage of initial activity remaining after dry and wet (100 mM phosphate buffer, pH 7.5) storage at room temperature. [d] TO hydrolysis and LA esterification activities after being used for 20 cycles of 24 h. [e] Propylsiloxylated-PHOMS. [f] Hexylsiloxylated-PHOMS. [g] 3-Gluconamidopropylsiloxylated-composite. Adsorbates with lipase protein loads of ∼20 mg g^{-1} were prepared and then fabricated into composites using 25% w/w of adsorbate, 55% w/w of PDMS, and 19% w/w of 4:1 PDES−PAPES crosslinker, and the 100−300 μm granule fractions employed. Abbreviations: LSC, lipase−silicone composite.

produced high activity biocomposites, indicating that the technique may be usefully extended to other enzymes. Physicochemical studies of the enzyme−PHOMS adsorbates and the catalytic biocomposites and further refinements to the polymers are currently in progress.

Experimental Section

Materials. Lipases from *C. rugosa*, porcine pancreas, *P. cepacia*, *R. arrhizus*, and *Mucor javanicus*, octyl β-D-glucopyranoside, Triton X-100, 4-nitrophenyl butyrate (4-NPB), and triolein (TO) were purchased from Sigma Chemical Co., UK. Lipases from *Aspergillus niger*, *P. fluorescens*, *P. cepacia*, *Penicillium roquefortii*, *M. meihei*, and *C. antartica* and lauric acid and octan-1-ol were obtained from Fluka Chemical Co., UK. PMHS (of M_r 1500−1900 and 1900−2200), phenyltriethoxysilane, propyltrimethoxysilane, dimethyldiethoxysilane, 3-aminopropyl)triethoxysilane, silanol-terminated poly(dimethylsiloxane) (PDMS, of M_r 550, 1750, 4200, 18 000, and 26 000 (5, 1.1, 0.85, 0.2, and 0.1% silanol contents, respectively), poly(diethylsilicate) (PDES, 41% w/w SiO_2), tin(II) 2-ethylhexanoate (TOC), and zinc 2-ethylhexanoate (ZOC) were purchased from ABCR GmBH & Co. KG, Karlsruhe, Germany.

Analytical Techniques. HPLC was carried out on using a LDC Milton Roy CM4000 pump connected to a Spectra Physics SP8450 UV/vis detector, LDC Marathon autosampler and an HP 35900 Chemstation, or a Waters 510 pump system connected to a Waters 717 Autosampler, an ACS Light Scattering detector and a Waters data collection and integration station, or a Varian 9012 ternary pump connected to a Spectra Physics RI detector and a Spectra Physics SP4290 Integrator module. Analyses were performed using a Hichrom RPB5 column (5 μm, 0.46 × 15 cm) eluted with water−acetonitrile or water−methanol, 40 °C, or a Chiracel OJ column (5 μm, 0.46 × 25 cm) eluted with hexane−propan-2-ol−methanol, 30 °C. GC was performed on a Varian Star 3400CX capillary GC equipped with an autosampler and FID detector and connected to a Varian Star data acquisition and analysis station. Analyses were performed on a 30 m SPB-1 column (0.25 mm diameter, 0.25 μm film thickness) or SPB-5 column (0.50 mm diameter, 0.5 μm film thickness) with helium as carrier gas. Analyses used an injection temperature of 250 °C, a split ratio of 50 or 100, a total flow of 30 mL/min, a detector temperature of 250 °C, and a temperature ramp of 70−300 °C over 20−30 min. UV−vis spectrometry was carried out on Kontron 930 and Perkin-Elmer Lambda-2 spectrometers, equipped with thermostated, stirred cells.

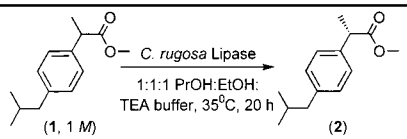
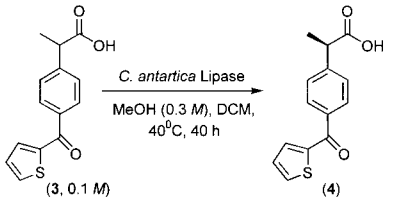
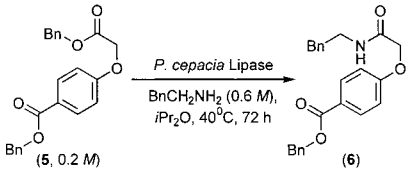
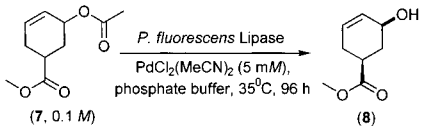
Preparation of PHOMS. (a) DMDO Method. A variation of the reported procedure,[7] was used: polymethylhydrogensiloxane (PMHS, 40 g in 500 mL of acetone) was added to ice-cold dimethyldioxirane (DMDO, 10 L of 80−90 mM in acetone) over 20 min, and the solution was stirred for 3 h and then concentrated at 20 °C to 10 g L^{-1}. This was evaporated to dryness at 20 °C, or concentrated to a gel, after which this was suspended in water, washed with 9:1 water−propan-2-ol, and stored wet (40−55% w/w solids). The silanol contents of the soluble material, the gel, and the dry solid were 11.4−12.3, 9.4−10.3, and 6.6−7.4 mmol g^{-1}, respectively.

(b) Acetone Method. PMHS (500 g in 500 mL of acetone) was added over 2 h at room temperature to a stirred mixture of zinc chloride (20 g), zinc 2-ethylhexanoate (10 g), and acetone (3 L). After 20 h the solution was concentrated at 20 °C, diluted with 3:1 propan-2-ol−water (1.5 L), and hydrolyzed with sodium hydroxide (2.3 L, 5.0 M aqueous, room temperature, 2 h), and the silanolate salt was decomposed with HCl (5.0 M aqueous, to pH 4−6, over 1 h). The suspension was filtered, washed with 9:1 water−propan-2-ol (6 × 1 L), and the PHOMS was stored wet (55−60% w/w solids, 8.8−10.7 mmol silanol g^{-1}). Washing with propan-2-ol, then cyclohexane, followed by drying at room temperature gave a polymer with 6.0−7.1 mmol silanol g^{-1}.

Lipase−PHOMS Immobilizates. Stock solutions of lipases were prepared by dissolving/suspending the enzyme (equivalent to approximately 5 mg of lipase protein) in ice-cold hexametaphosphate buffer (1−50 mL, 50 mM, pH 7, containing 25 mM each of calcium and magnesium acetates, 1 h), centrifuging (10000*g*, 10 min), and discarding the solid residue. Enzyme stock was added to stirred, ice-cold PHOMS (20 g, 51−57% w/w solids, 9.3−10.1 mmol SiOH g^{-1}), propyl-PHOMS (20 g, 52% w/w solids, 5.9 mmol SiOH g^{-1}), or hexyl-PHOMS (20 g, 57% w/w solids, 6.1 mmol SiOH g^{-1}) in 9:1 buffer−

(26) We have routinely fabricated lipase−PHOMS−silicone catalysts on a multikilo scale, and have estimated the costs of producing monoliths, granulates, coated particulates, and foams to be in the range of $20−35 per kilo of composite. The prices of commercial food and catalyst grade lipases range over $100−2000 per kilo. For medium to high catalytic density immobilizates (5−15% w/w lipase loading), the additional cost of immobilization is about 9−30%. Considering that the activities and long-term stabilities of the composites can exceed those of the native enzymes by 1−50-fold and 10−30-fold respectively, leading to performance improvements of 10−1500-fold, it can be seen that the economics are rather attractive.

Table 3. Representative Synthetic Applications of Lipase−PHOMS−Silicone Composite (LPSC) Biocatalysts

Reaction, Biocatalyst and Conditions	Catalyst Type	Long Term Performance of Biocatalyst							
		First Cycle of Use				10th Cycle of Use			
		Activity[a]	Conversion (%)	E	%*ee*	Activity[a]	Conversion (%)	E	%*ee*
 (1, 1 *M*) (2)	*C. rugosa* Lipase B	24	39	60	94	-	-		-
	ChiroCLEC™-CR	91	46	170	97	80	39	60	94
	Silicone Composite	108 (4.6, 1.2)	48	146	96	97 (-, 1.2)	44	61	93
 (3, 0.1 *M*) (4)	Free Enzyme	0.66	43	10	54	0.20	12	10	11
	Novozym® 435	0.89	69	10	98	0.72	54	10	76
	Silicone Composite	2.71 (4.1, 3.0)	73	10	99	2.63 (13.2, 3.7)	68	11	98
 (5, 0.2 *M*) (6)	Sigma Enzyme	0.21	40	-	-	0.08	18	-	-
	Amano PS-30	0.37	74	-	-	0.31	64	-	-
	Silicone Composite	1.40 (6.7, 3.8)	96	-	-	1.33 (16.6, 4.3)	91	-	-
 (7, 0.1 *M*) (8)	Biocatalysts L056P	0.11	58	5.0	61	0.06	23	3.9	57
	Silicone Composite	0.58 (5.3)	93	29	86	0.52 (8.7)	88	21	83

[a] Units of μmol h^{-1} mg^{-1}. Figures in parentheses indicate the relative specific activities of the silicone composites as compared with the free enzymes and commercial immobilizates. Adsorbates with lipase protein loads of 1−50 mg g^{-1} were prepared and then fabricated into composites using 25% w/w of adsorbate, 55% w/w of PDMS, and 19% w/w of 4:1 PDES−PAPES crosslinker. Free enzyme, commercial immobilizate, or 100−300 μm biocomposite was added to the substrate solution, the mixture stirred at 300−400 rpm, filtered, and the catalyst washed with ice-cold acetone and reused. Conditions: (a) **1** to **2**: 1 M substrate in buffer (0.3 M, pH 7.5, containing 30 mM $CaCl_2$ and 10 mM $MgCl_2$), 35 °C, with a lipase equivalent of 25 mg mL^{-1}. It was not possible to reuse the free enzyme because of difficulty in recovery from the mixed aqueous-organic reaction medium; (b) **3** to **4**: 0.1 M substrate and 0.3 M methanol in dichloromethane, 40 °C, with a lipase equivalent of 50 mg mL^{-1}; (c) **5** to **6**: 0.2 M **5** and 0.5 M phenylethylamine in diisopropyl ether, 40 °C, with a lipase equivalent of 50 mg mL^{-1}; (d) **7** to **8**: 0.1 M substrate in 3:7 ethanol−buffer (0.1 M, pH 7.5, containing 50 mM $CaCl_2$ and 25 mM $MgCl_2$), with 5 mM bis(acetonitrile)palladium(II) chloride (5 mol%) and a lipase equivalent of 100 mg mL^{-1}. Reactions were followed by HPLC.

propan-2-ol (20−50 mL). After 15 min, hexametaphosphate buffer (100−200 mL, 0.25 M, pH 7) was added and stirring continued for 2 h. The suspension was filtered, and the gel was washed with ice-cold buffer, ice-cold acetone, followed by ice-cold pentane (3 × 30 mL of each), and the cake was dried at room temperature for 2 h. The supernatant and aqueous washes were assayed for protein and lipase activity by Lowry, 4-NPB hydrolysis, and UV. In the *C. rugosa* loading experiments, PHOMS (1 g) was contacted with crude/purified lipase stock (1−40 mL) containing a lipase protein equivalent of 5 mg mL^{-1}, according to the general procedure, and the supernatants and immobilizates assayed using 4-NPB.

Lipase−PHOMS−Silicone Composites. Lipase−PHOMS (6 g) was stirred with ST-PDMS (7 g each of M_r 1750 (1.1% SiOH) and M_r 26 000 (0.1% SiOH)), this was mixed with PDES (5 g, 41% w/w solids) and TOC (0.15 g), and the dispersion was poured onto glass and allowed to cure at room temperature for 5 h, to give a translucent, flexible biocomposite sheet (22−23 g, 4−6 mm thick). This was ground under ice-cold heptane in a homogenizer, and the granules were washed with pentane (3 × 30 mL), dried in air at room temperature for 1 h, and then sieved to recover the 50−200, 100−300, 300−500, and 500−1,000 μm fractions. Hydrophilic composites were made using a mixture of PDES (20−80% w/w) and PAPES (20−80% w/w) as cross-linker. The granulated composite (10 g) was then treated with δ-gluconolactone (100 mL of 10% w/v in 4:1 water−propan-2-ol) at room temperature for 10 h, filtered, washed with 4:1 water-propan-2-ol and then heptane (3 × 30 mL of each), and dried in air at room temperature for 1 h. The enzyme loadings of the composites were 24−27% of those of the primary lipase−PHOMS immobilizates. For leaching trials, composite (10 g of 50−200 μm) was stirred with buffer (100 mL, 50 mM) or 4:1 buffer-propan-2-ol, at room temperature for 5 days, followed by filtering and assaying the filtrate by Lowry and 4-NPB hydrolysis. The loss of 0.1−0.6% of the immobilized protein was detected.

Synthetic Applications. The reactions were conducted according to the published procedures,[4d,24] with some modifications as noted below. Adsorbates with lipase protein loads of 1−50 mg g^{-1} were prepared using 10 mg mL^{-1} (purified enzyme) or 30 mg mL^{-1} (crude enzyme) lipase stocks and then encapsulated (25% w/w of adsorbate, 55% w/w of PDMS mix, and 19% w/w of 4:1 PDES−PAPES cross-linker mix) according to the general procedure. Biocatalyst (free enzyme, commercial immobilizate or 100−200 μm biocomposite) was added to the reaction solution, the mixture was stirred (300−400 rpm) for 20−96 h and then filtered, and the catalyst was washed with ice-cold acetone and reused. The conditions were as follows:

(a) 1 to 2: 1 M **1** in 1:1:1 propan-2-ol−ethanol−TEA buffer (0.3 M, pH 7.5, containing 30 mM calcium chloride and 10 mM magnesium chloride), 35 °C, with a lipase equivalent of 25 mg mL^{-1}.

(b) 3 to 4: 0.1 M **3** and 0.3 M methanol in dichloromethane, 40 °C, with a lipase equivalent of 50 mg mL^{-1}.

(c) 5 to 6: 0.2 M **5** and 0.6 M phenylethylamine in diisopropyl ether, 40 °C, with a lipase equivalent of 50 mg mL^{-1}.

(d) 7 to 8: 0.1 M **7** in 3:7 ethanol−phosphate buffer (0.1 M, pH 7.5, containing 50 mM calcium chloride and 25 mM magnesium chloride), with 5 mM bis(acetonitrile)palladium(II) chloride (5 mol %) and a lipase equivalent of 100 mg mL^{-1}. For (b) and (c), the catalysts were washed with dichloromethane saturated with phosphate buffer (0.1 M, pH 7.5, containing 30 mM calcium chloride and 10 mM magnesium chloride) between each recycle. The reactions were followed by RP-HPLC or chiral HPLC and product identities confirmed by comparison with standards.[4d,23]

Determination of Enzyme Activities. Lipase activities were determined from the hydrolysis of soluble 4-nitrophenyl butyrate (4-NPB), from the hydrolysis of emulsified triolein (TO), and by esterification of lauric acid (LA) with octan-1-ol (OcOH) in isooctane. The various activities of the native enzymes, as determined below, are given in Table 3.

4-NPB Hydrolysis in a Monophasic System. The hydrolytic activities of the biocatalysts toward soluble 4-NPB were determined using the substrate (5 mM) dissolved in Tris buffer (50 mM, pH 7.5, containing 25 mM calcium acetate, 10 mM magnesium acetate, and 15% v/v acetone), 30 °C. The hydrolysis rate was determined spectrophotometrically at 346 nm (Kontron 930 spectrometer or Perkin-Elmer Lambda-2 spectrometer). Assays with the nonimmobilized lipases were carried out by adding enzyme stock (50 μL of 5−25 mg mL^{-1}) in Tris buffer (50 mM, pH 7.5, containing 25 mM calcium acetate, 10 mM magnesium acetate, and 10% v/v propan-2-ol) to the substrate (1 mL, stirred at 200 rpm) preequilibrated in the assay cuvette, and following the absorbance over 1−10 min. In the case of the immobilized enzymes, the biocatalyst (8−80 mg of 50−200 μm or 100−300 μm powder) was stirred (200 rpm) in Tris buffer (0.2 mL of 50 mM, pH 7.5, containing 25 mM calcium acetate, 10 mM magnesium acetate, and 10% v/v propan-2-ol) for 15 min, the preequilibrated substrate solution (4 mL) added, and the reaction allowed to proceed for 1−10 min. A sample (1 mL) of reaction mixture was taken and quenched with methanol (10 mL) and filtered (2 mL, 0.5 μm nylon centrifuge filter, 5,000 g, 1 min), and the absorbance of the supernatant was determined.

Hydrolysis of TO in an Emulsion System. The hydrolytic activities of the biocatalysts toward a TO emulsion were determined using TO (150 mM) homogenized in Tris buffer (5 mM, pH 8, containing 25 mM Triton X-100, 30 mM calcium acetate, 10 mM magnesium acetate, and 2% w/v gum arabic), 30 °C. Reactions were followed titrimetically (Metrohm 718 STAT Titrino Titrator, 25 mM or 50 mM NaOH titrant). Assays were performed by adding the native enzyme stock (5 mL of 2−20 mg mL^{-1} in 50 mM Tris buffer, pH 7.5, containing 25 mM calcium acetate and 10 mM magnesium acetate) or immobilized enzyme (50−500 mg of 50−200 μm or 100−300 μm powder) suspended in Tris buffer (5 mL of 50 mM, pH 7.5, containing 25 mM calcium acetate, 10 mM magnesium acetate and 10% v/v propan-2-ol) to 50 mL of stirred (300−400 rpm) substrate solution, and titrating for 10−40 min.

Esterification of LA with OcOH in Isooctane. The esterification activities of the catalysts were determined using LA (50 mM) and OcOH (100 mM) dissolved in isooctane saturated with Tris buffer (50 mM, pH 7.5, containing 20 mM calcium chloride and 10 mM magnesium chloride), 30 °C. Reactions were performed by adding preequilibrated reaction solution (5 mL) to a preequilibrated (15 min, 30 °C) suspension of the nonimmobilized lipase (10−200 mg of lyophilizate, sonicated for 10 min) or the immobilized enzyme (10−200 mg of 50−200 μm or 100−300 μm powder) in buffer-saturated isooctane (0.5 mL), sonicating (nonimmobilized enzymes only, 5 min), and stirring (300−400 rpm) for 20−180 min. Samples of the reaction mixtures were taken, centrifuged (10000*g*, 5 min), and then analyzed by RP-HPLC (Waters 510 Pumps, Waters 717 Autosampler, ACS Light Scattering detector, Waters DCI Collection Module and software, 0.46 × 15 cm Hichrom RPB-5 ODS2 column, 30 °C, eluted with 65:25:9.5:0.5 acetonitrile−dichloromethane−tetrahydrofuran−acetic acid), or GC (Varian Star 3400CX capillary GC with autosampler, 0.25 μm × 0.25 mm × 15 m DB-1 capillary column, split injection at 200 °C, flow rate of 30 mL min^{-1}, FID detector at 250 °C, ramp of 75−300 °C over 30 min).

Storage Stabilities. The lipase−PHOMS−silicone composites (100 mg of 50−200 μm powder) were stored either dry (nondesiccated) or wet (in 10 mL of 100 mM phosphate buffer, pH 7.5, containing 25 mM calcium acetate) at room temperature and under ambient lighting for a period of 6 months. The initial activities and activities remaining at the end of the storage period were determined using the standard 4-NPB assay.

Operational Stabilities. The stabilities of lipase−PHOMS adsorbates and composites during the repetitive, batchwise hydrolysis of TO or esterification of LA with OcOH were performed as follows:

(a) TO Hydrolysis. Lipase−PHOMS adsorbate or lipase−PHOMS−silicone catalyst (20−200 mg of adsorbate powder or 50−500 mg of composite as 50−200 μm or 100−300 μm fraction) was suspended in 100 mL of TO emulsion (150 mM, homogenized in Tris buffer, 5 mM, containing 25 mM Triton X-100, 30 mM calcium acetate, 10 mM magnesium acetate, and 2% w/v gum arabic), 30 °C and the reaction allowed to proceed for 24 h with automatic titration (setpoint at pH 7.5). For the adsorbates, the suspension was then centrifuged (12000*g*, 10 min), the catalyst pellet washed by resuspension and centrifugation (3 × 5 mL of 50 mM Tris buffer, pH 7.5, containing 10% v/v propan-2-ol), a new batch of TO substrate added, and the process repeated. For the composites, the aqueous phase was removed using a 10 μm stainless steel suction filter tube, the catalyst was washed (3 × 5 mL of 50 mM Tris buffer, pH 7.5, containing 10% v/v propan-2-ol), the washings were suction-filtered off, and the reaction was repeated. The recycle operations were performed a total of 20 times and the TO hydrolysis rates of the first and last runs compared.

(b) LA + OcOH Esterification. Lipase−PHOMS adsorbate or lipase−PHOMS−silicone catalyst (20−100 mg of adsorbate powder or 50−250 mg of composite as 50−200 μm or 100−300 μm fraction) was suspended in LA + OcOH reaction mixture (20 mL of 50 mM LA, 100 mM OcOH, in buffer-saturated isooctane), 30 °C, and the reaction allowed to proceed for 24 h with stirring (300−400 rpm). The organic phase was removed by centrifugation (12000*g*, 10 min) or by filtration through a 5 μm stainless steel suction filter tube, the catalyst was washed (3 × 10 mL of dry isooctane), the washings were removed by centrifugation or filtration, a new batch of reaction mixture was added, and the process was repeated. Every four recycles, the catalysts were washed with 2:1 isooctane−acetone (3 × 5 mL), so as to avoid the build up of water in the catalysts. Recycling was performed a total of 20 times, and the initial and final esterification rates of the first and last runs were compared.

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Supporting Information Available: Additional analytical techniques (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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