ORGANIC LETTERS

2001
Vol. 3, No. 13
2025−2028

Enantioselective Aminolysis of an α-Chloroester Catalyzed by *Candida cylindracea* Lipase Encapsulated in Sol–Gel Silica Glass

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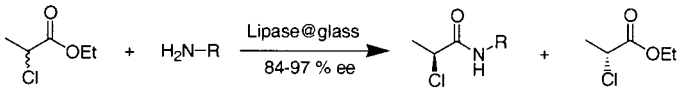
Received April 16, 2001

ABSTRACT



R is *p*-MeOC$_6$H$_4$, *p*-tolyl, PhCH$_2$, *n*-Bu, Cy

Lipase from *Candida cylindracea* (CcL) encapsulated in porous silica glass by a sol–gel method catalyzes enantioselective aminolysis of ethyl 2-chloropropionate. A silica matrix enhances the enzyme activity, i.e., improves the yield. The scope and limitations of the aminolysis reaction were investigated, and dynamic kinetic resolution of the ester was achieved. Encapsulated lipase remains active when used repeatedly. Encapsulation much improves the chiral discrimination by lipase and makes this enzyme even more useful in organic chemistry.

Glasses made of silica and its various organic derivatives may be prepared at room temperature by the sol−gel method, that is, by condensation−polymerization of suitable alkoxides.[1] Mild reaction conditions allow encapsulation in these glasses of enzymes, catalytic antibodies, and other proteins.[2] The resulting biocomposite materials hold great promise as sensors and heterogeneous catalysts, but the fulfillment of this promise requires study of the interactions between the protein and the surrounding glass matrix. This study has only begun,[3] and much remains to be learned about the various effects of the matrix on activity and stability of encapsulated biomolecules.

Lipases, obtained from various organisms, are the most useful enzymes available to organic chemists today.[4] They catalyze esterification, transesterification, amidation, transamidation, and hydrolysis of many substrates. Because of this great utility of lipases, their encapsulation in sol−gel glasses has been studied more than that of any other enzymes.[5] Research by Reetz and co-workers showed that the enzyme activity and stability can be adjusted, even improved, by varying the hydrophobicity of the matrix.[5a] Making an enzyme into a heterogeneous catalyst is very advantageous in synthetic practice, and encapsulated lipases can be purchased.

The main advantage of lipases as catalysts in organic chemistry—their great enantioselectivity—has barely been studied in sol−gel glasses.[6] This is the subject of our Letter. Lipase from C*andida cylindracea* catalyzes enantioselective aminolysis of esters.[7] We compare the effectiveness of this

(1) (a) Husing, N.; Schubert, U. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 22−45. (b) Dunn, B.; Zink, J. I. *Chem. Mater.* **1997**, *9*, 2280−2291. (c) Avnir, D. *Acc. Chem. Res.* **1995**, *28*, 328−334.

(2) (a) Bhatia, R. B.; Brinker, C. J.; Gupta, A. K.; Singh, A. K. *Chem. Mater.* **2000**, *12*, 2434−2441. (b) Avnir, D.; Braun, S.; Lev, O.; Ottolenghi, M. *Chem. Mater.* **1994**, *6*, 1605−1614.

(3) (a) Badjić, J. D.; Kostić, N. M. *Chem. Mater.* **1999**, *11*, 3671−3679. (b) Gottfried, D. S.; Kagan, A.; Hoffman, B. M.; Friedman, J. M. *J. Phys. Chem. B* **1999**, *103*, 2803−2807. (c) Chen, Q.; Kenausis, G. L.; Heller, A. *J. Am. Chem. Soc.* **1998**, *120*, 4582−4585.

(4) (a) Lopez-Serrano, P.; Wegman, M. A.; van Rantwijk, F.; Sheldon, R. A. *Tetrahedron: Asymmetry* **2001**, *12*, 235−240. (b) Lau, R. M.; van Rantwijk, F.; Seddon, K. R.; Sheldon, R. A. *Org. Lett.* **2000**, *2*, 4189−4191. (c) Theil, F. *Chem. Rev.* **1995**, *95*, 2203−27.

(5) (a) Reetz, M. T.; Wenkel, R.; Avnir, D. *Synthesis* **2000**, *6*, 781−783. (b) Reetz, M. T. *Adv. Mater.* **1997**, *9*, 943−954. (c) Reetz, M. T.; Zonta, A.; Simpelkamp, J. *Biotech. Bioeng.* **1996**, *49*, 527−534. (d) Reetz, M.; Zonta, A.; Simpelkamp, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 301−303. (e) Furukawa, S.; Ono, T.; Ijima, H.; Kawakami, K. *Kagaku Kogaku Ronbunshu* **2000**, *26*, 882−886.

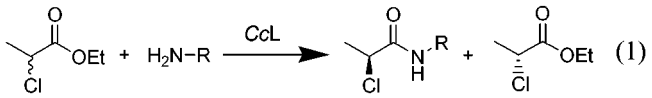
(6) Jaeger, K.-E.; Schneidinger, B.; Rosenau, F.; Werner, M.; Lang, D.; Dijkstra, B. W.; Schimossek, K.; Zonta, A.; Reetz, M. T. *J. Mol. Catal. B: Enz* **1997**, *3*, 3−12.

10.1021/ol015989o CCC: $20.00
Published on Web 06/07/2001

enzyme when suspended in the reaction mixture and when encapsulated in sol−gel silica powder that is suspended in the reaction mixture. We are interested in the stability and catalytic activity of the encapsulated enzyme, enantioselectivity of the aminolysis reaction, and recyclability of this catalyst. Our results show a considerable advantage of the encapsulated over the free enzyme.

Objective of this Study. We examine the effect of silica matrix on the stability of encapsulated *C. cylindracea* lipase and its enantioselectivity. Our very recent study of catalysts other than lipase showed that the silica matrix may lower the enantioselectivity of sulfoxidation by indirectly enhancing the "background" reaction, which is not enantioselective.[8] Clearly, enantioselectivity of reactions in the silica matrix cannot be taken for granted.

Choice of Enantioselective Reaction. Crude *C. cylindracea* lipase (*Cc*L) suspended in an organic solvent catalyzes the reaction of racemic esters with various amines as shown in eq 1. Yield and enantioselectivity generally are



(1)

good.[7] We use this known reaction to study the possible effects of organically modified sol−gel silica glass on enzyme stability, reaction yield, and enantioselectivity. For kinetic resolution of the racemic ester, a 2:1 ratio of ester to amine should be used. Ideally, the faster-reacting enantiomer of the ester would be converted to a single enantiomer of the amide, and the slower-reacting enantiomer of the ester would remain. When the ester-to-amine ratio is greater than 2:1, the amine is the limiting reagent; when this ratio is lower than 2:1, the faster-reacting enantiomer of the ester is the limiting reagent.

Encapsulation of the Lipase. The enzyme was encapsulated into hydrophobic sol−gel glass using a modification of the published method.[5c] The water-soluble fraction of the enzyme (designated ws*Cc*L) was extracted from the purchased crude enzyme.[9] A 5:1 molar ratio of trimethoxypropylsilane to tetramethoxysilane was used for the sol−gel method. Experimental procedure can be found in the Supporting Information.

Enantioselective Aminolysis of Ethyl 2-Chloropropionate by *p*-Anisidine at 60 °C. An aminolysis reaction in the presence of the water-soluble fraction of *Cc*L (see Table 1, entry 1) after 24 h gave a minute yield, but good enantioselectivity. After several days, the yield increased somewhat, but the enantioselectivity almost vanished (entries 2 and 3). Encapsulated *Cc*L (designated *Cc*L@glass) yielded much more amide than the free enzyme did, and with excellent enantioselectivity. Entries 4 and 1 show that encapsulated lipase under the given conditions is more reactive than free lipase but that the reaction ceases after ca. 24 h.

Table 1. Enantioselective Aminolysis of Ethyl 2-Chloropropionate by *p*-Anisidine Catalyzed by *C. cylindracea* Lipase at 60 °C

entry	catalyst	ester:amine	time (h)	yield (%)	ee (%)
1	ws*Cc*L[a]	2:1	24	1.3	85
2			48	6.0	6.0
3			217	21	2.0
4	*Cc*L@glass[b]	2:1	24	64	94
5		5:1	30	54	96
6		1:1	24	62	86
7		1:2	25	116[c]	71[c]
8	*Cc*L@glass[d]	2:1	26	4	74

[a] Water-soluble fraction of *C. cylindracea* lipase. [b] *C. cylindracea* lipase encapsulated into sol−gel silica glass. [c] Slower-reacting enantiomer of ester also reacts under these conditions (see text). [d] After 24 h at 60 °C.

Entries 4−7 show the effects of the reactant ratio. Lowering the ester-to-amine ratio (entries 6 and 7) raises the yield but lowers the enantioselectivity. A yield greater than 100%, accompanied by a decline in enantioselectivity (entry 7), shows that the slower-reacting enantiomer of the ester also reacts when the amine is present in large excess. Since amine was never in excess in our other experiments, this anomaly was avoided.

To test the recyclability, the catalyst was repeatedly recovered from the reaction mixture, washed with CCl_4 and acetone, and reused. As Figure 1 shows, after just three 24

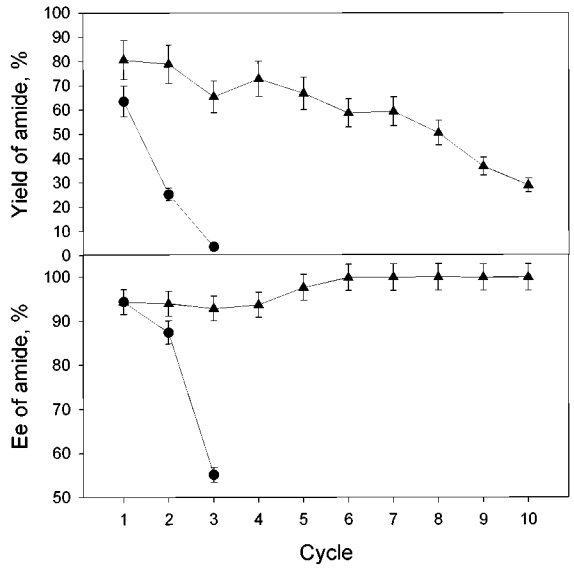


Figure 1. Effect of recycling of *C. cylindracea* lipase encapsulated into sol−gel silica glass on the yield of amide (top) and enantiomeric excess of its (*S*)-enantiomer (bottom). All the experiments lasted for 24 h, and the ratio of racemic ester to amine was 2:1. Temperature was 60 (●) and 25 (▲) °C.

(7) (a) Gotor, V. *Bioorg. Med. Chem.* **1999**, *7*, 2189−2197. (b) Gotor, V.; Brieva, R.; Gonzalez, C.; Rebolledo, F. *Tetrahedron* **1991**, *47*, 9207−14.

(8) Kadnikova, E. N.; Kostić, N. M. *J. Non-Cryst. Solids* **2001**, *283*, 63−68.

(9) Lewis, G.; Bevan, J.; Rawas, A.; McMichael, P.; Wisdom, R.; McCague, R.; Watson, H.; Littlechild, J. *Acta Crystallogr., D: Biol. Crystallogr.* **1997**, *D53*, 348−351.

h cycles at 60 °C the yield drops to single digits and the enantioselectivity declines.

After the encapsulated enzyme was heated in CCl_4 at 70 °C for 14 days, it was filtered off, and the organic phase was thoroughly extracted with water. The Bradford test showed no protein in the extract. Given the sensitivity of this assay, at most 0.10−0.20% of the encapsulated enzyme might have leaked from the silica. When the encapsulated enzyme was kept at 60 °C for 24 h prior to the addition of the reactants (entry 8), both the yield and the enantioselectivity declined from the values obtained with the fresh catalyst (entry 4). These experiments show that the decline in the performance of encapsulated lipase evident in Figure 1 is due to thermal inactivation of the enzyme at 60 °C, not to the leakage of the enzyme from the glass matrix.

Enantioselective Aminolysis of Ethyl 2-Chloropropionate by *p*-Anisidine at 25 °C. Both water-soluble (Table 2, entries 1−3) and encapsulated (Table 2, entries 4−6) forms of lipase afford higher yields and higher enantioselectivities at 25 °C than at 60 °C (Table 1, entries 1−3 and 4, respectively). Moreover, at 25 °C encapsulated enzyme is a much more efficient catalyst than the free enzyme (Table 2, entries 4−6 vs 1−3). When encapsulated lipase was kept at 25 °C for 48 h before addition of the reactants (Table 2, entries 7−9), the yield declined somewhat, but the enantioselectivity remained high.

Table 2. Enantioselective Aminolysis of Ethyl 2-Chloropropionate by *p*-Anisidine Catalyzed by *C. cylindracea* Lipase at 25 °C

entry	catalyst	time (h)	yield (%)	ee (%)
1	ws*Cc*L[a]	24	7	94
2		48	16	87
3		310	31	83
4	*Cc*L@glass[b]	24	81	94
5		48	89	94
6		197	100	88
7	*Cc*L@glass[c]	25	58	98
8		44	67	94
9		235	79	93

[a] Water-soluble fraction of the enzyme. [a] The enzyme encapsulated into sol−gel silica glass. [c] After 48 h at 25 °C.

As Figure 1 shows, the encapsulated enzyme remains quite efficient after many cycles at 25 °C. In fact, the relative decline in yield after 10 cycles at 25 °C is still less than that after just two cycles at 60 °C. Moreover, enantioselectivity remains very high after 10 cycles at 25 °C. This good behavior at room temperature makes encapsulated lipase a convenient catalyst for synthesis.

Enantioselective Aminolysis of Ethyl 2-Chloropropionate by Other Amines. We also studied several other amines in the aminolysis reaction. The results are shown in Table 3. Both *p*-toluidine (entries 1−2) and benzylamine (entries 3−4) afforded more amide, with better enantioselectivity, at 25 °C than at 60 °C. These results are consistent with the thermal inactivation of enzyme at a higher temperature, as we also found for *p*-anisidine (see above). Results with more volatile aliphatic amines (entries 5−12) were virtually the same at 25 °C and 5 °C.

Table 3. Enantioselective Aminolysis of Ethyl 2-Chloropropionate by Amines RNH_2 Catalyzed by *C. cylindracea* Lipase

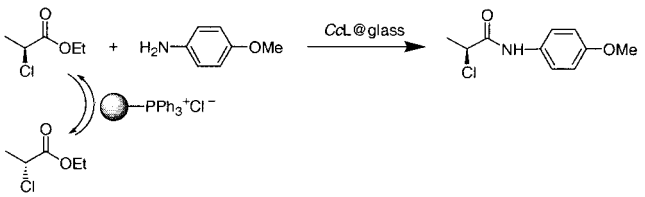
				*Cc*L@glass[a]		ws*Cc*L[b]	
entry	R	temp (°C)	time (h)	yield (%)	ee (%)	yield (%)	ee (%)
1	*p*-tolyl	60	24	28	76	0.6	37
2		25	127	92	86	2.4	5
3	$PhCH_2$	60	24	20	95	1.4	6
4		25	170	43	97	4.5	62
5	allyl	25	4	25	3[c]	2.1	0[c]
6		5	12	23	3[c]	1.5	0[c]
7	*n*-Bu	25	6	40	88	1.5	39
8		5	12	38	95	1.3	96
9	Cy	25	3	58	84	2.0	62
10		5	3	22	77	1.5	37
11	*t*-Bu	25	4	51	13	1.7	0
12		5	12	50	10	1.5	0

[a] The enzyme encapsulated into sol−gel silica glass. [b] Water-soluble fraction of the enzyme. [c] Estimated from the enantiomeric excess of starting material after the reaction (see Supporting Information).

Good-to-excellent yields of the corresponding amide, with excellent enantioselectivities, were achieved for *p*-toluidine, benzylamine, *n*-butylamine, and cyclohexylamine. The comparison of the experiments in the presence of *Cc*L@glass and ws*Cc*L clearly demonstrates the superiority of encapsulated over free enzyme.

Dynamic Kinetic Resolution of Ethyl 2-Chloropropionate into *N*-*p*-Methoxyphenyl-2-chloropropionamide via Aminolysis in the Presence of the Chloride Ion. Most of the experiments described above were carried out under kinetic resolution conditions, namely, with a 2:1 ratio of racemic ester to amine. The two main disadvantages of kinetic resolution are that one-half of the substrate remains unspent and that enantioselectivity decreases as the reaction progresses because the slower-reacting enantiomer becomes dominant in the substrate. The racemic substrate can be completely converted to a single enantiomer of the product

Scheme 1. Dynamic Kinetic Resolution of Ethyl 2-Chloropropionate via Aminolysis Catalyzed by Encapsulated *C. cylindracea* Lipase in the Presence of Triphenylphosphonium Chloride Immobilized on Merrifield Resin



in the presence of a suitable agent, which racemizes the substrate much more rapidly than the product. Under dynamic kinetic resolution, shown in Scheme 1, enantioselectivity remains constant throughout the reaction.[10]

The chloride salt of the triphenylphosphonium cation bound to the Merrifield resin effectively racemizes α-chloroester.[11] We prepared this immobilized catalyst by a published procedure.[12]

As Figure 2 shows, the reaction in the absence of this catalyst slows down after reaching ca. 50% conversion; the enantiomeric excess of the product also declines sharply around this point. The final yield of amide is 70%, and its enantiomeric excess is 71%. Comparison of this result to the results obtained under kinetic resolution conditions (Table 2, entries 4−6) again shows the importance of the reactant ratio for efficient resolution.

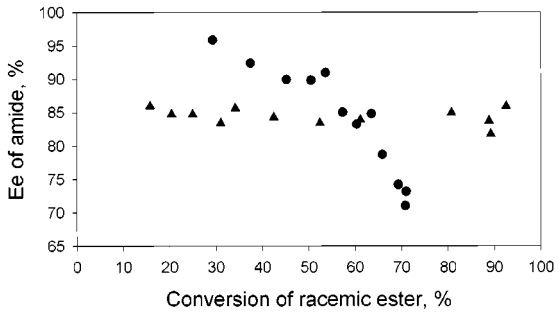


Figure 2. Enantioselective aminolysis of ethyl 2-chloropropionate by an equimolar amount of *p*-anisidine catalyzed by encapsulated *C. cylindracea* lipase in the presence (▲) and in the absence (●) of the racemizing agent, triphenylphosphonium chloride immobilized on Merrifield resin. Reaction mixtures were sampled after 3, 6, 12, 18, 24, 36, 48, 72, 126, 192, 240, and 312 h. The 12 points in each set correspond to the incremental increase in conversion from left to right.

The reaction in the presence of the racemizing agent proceeds to very high conversion. As Figure 2 shows, the enantiomeric excess of amide stays constant throughout. The final yield of amide now is 92%, and its enantiomeric excess is 86%. The small difference between the initial enantioselectivity (94%, in Table 2, entry 1) in the absence and the constant enantioselectivity (86%) in the presence of the phosphonium chloride can be attributed to partial racemization of the product. Fortunately, racemization of the product is much slower than that of the substrate. To our knowledge, this is the first example of dynamic kinetic resolution of a racemic ester into an amide via aminolysis.

C. cylindracea lipase encapsulated into sol−gel silica glass catalyzes enantioselective aminolysis of an ester. Silica matrix stabilizes the enzyme and enhances its activity under the conditions used in our study. Yield and enantioselective excess of the product, an amide, depend on temperature, stoichiometry, and the substrate. The reaction yield is further improved under dynamic kinetic resolution. The method used in this study can be applied to other enantioselective reactions catalyzed by enzymes. Encapsulation in sol−gel glasses preserves chiral discrimination by lipase and extends the usefulness of this enzyme in organic chemistry.

Acknowledgment. We thank Prof. Jonathan M. J. Williams for a helpful suggestion and Dr. Kamel Harrata for help with optimization of chiral GC-MS conditions. This research was supported by the National Science Foundation.

Supporting Information Available: Experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) (a) Strauss, U. T.; Felfer, U.; Faber, K. *Tetrahedron: Asymmetry* **1999**, *10*, 107−117. (b) Stecher, H.; Faber, K. *Synthesis* **1997**, 1−16.

(11) Haughton, L.; Williams, J. M. J. *Abstracts of Papers*, 220th National Meeting of the American Chemical Society, Washington, DC, August 2000; American Chemical Society: Washington, DC, 2000; ORGN-112.

(12) Zheng, A.; Shan, D.; Shi, X.; Wang, B. *J. Org. Chem.* **1999**, *64*, 7459−7466.