Preliminary Communication

Preparation and Catalytic Performance of Lipases Encapsulated in Sol-Gel Materials

Katsuya Kato, Yuefa Gong, Takao Saito, and Yoshiyuki Yokogawa

Bio-functional Ceramics Group, Ceramics Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 2266-98 Anagahora, Simoshidami, Moriyama-ku, Nagoya 463-8560, Japan

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Three kinds of lipases (from Candida antarctica, Pseudomonas cepacia, and Pseudomonas fluorescens) were encapsulated in inorganic matrices by the sol-gel method in order to synthesize chiral compounds by kinetic resolution. Sol-gel lipases prepared with vinyltriethoxysilane had higher hydrolysis activity for 2-octyl acetate than those with other silane precursors: tetramethoxysilane, methyltrimethoxysilane, and propyltrimethoxysilane.

Key words: lipase; enantioselectivity; sol-gel; encapsulated; vinyltriethoxysilane

The sol-gel process is a method for preparing inorganic oxide matrices of metals and semimetals by direct hydrolysis and polycondensation of active monomeric precursors. Resulting matrices have large surface areas; high porosity, inertness, and stability in the presence of chemical and physical agents; and visible and UV optical clarity. The reactions occur at room temperature, which permits organic and bioorganic molecules to be trapped within the forming silica network. Biomolecules, which are strongly encapsulated within the matrix and cannot diffuse out, generally retain their activities, gain higher stability, and are able to react with ligands that diffuse into the highly porous matrix. Improved stability of the entrapped biomolecules and the physical and chemical properties of the matrix are major reasons to use sol-gels for the immobilization of biomolecules in general and for the encapsulation of enzymes in particular. The sol-gel chemical route to various materials therefore has been studied very intensively in recent years, resulting in the production of many biomolecules that have diverse applications.1-4

During the past decade, numerous articles have been published on the use of lipases as catalysts in organic synthesis. Reactions of commercial value catalyzed by lipases include hydrolysis, as well as esterification and transesterification in nonaqueous media. Lipases also have been used to resolve kinetically or to synthesize chiral products from racemic or prochiral compounds.5,6 Reetz et al. reported7,8 that lipases entrapped in hydrophobic sol-gel materials prepared from a mixture of tetramethoxysilane and propyltrimethoxysilane catalyze the hydrolysis of p-nitrophenyl propionate in aqueous solution well and that entrapped lipases could be recycled many times with little decrease in hydrolytic activity. However, less attention has been paid to the effects of the sol-gel encapsulation on lipase enantioselectivity. Very recently, Banjic et al. reported9 the enantioselective aminolysis of α-chloroester by Candida cylindracea lipase encapsulated in a sol-gel matrix prepared by a modification of Reetz’s procedure. There have been few reports on the reactivity and enantioselectivity of lipases encapsulated in sol-gel matrices. During our study of lipase immobilization on inorganic materials for the preparation of optically active compounds,10 we found that lipase encapsulated in a sol-gel matrix prepared from vinyltriethoxysilane had good reactivity and high thermal stability. Details of the reactions of the sol-gel lipases tested follow.

The test lipases were encapsulated in sol-gel matrices by modification of the method described previously.7,9 Vinyltriethoxysilane (1.14 g, 6 mmol) was added to a mixture of 400 μl of Candida antarctica lipase (SP 525 L, 0.8 mg protein/ml, 15,000 units/g), 100 μl of 1 N aqueous sodium fluoride, 200 μl of aqueous poly (vinyl alcohol) (4% w/w, mw 22,000), and 164 μl of distilled water in a test tube. After being vigorously stirred in a vortex mixer for 10 s, the mixture was shaken by hand for another 5 min. The sealed tube was kept at room temperature overnight. The material was air-dried at room temperature for 3 days and then ground in a mortar. After being shaken with distilled water, the solid material was filtered out, and washed with cold acetone and n-hexane. The gel was air-dried overnight and ground in a mortar, affording a sol-gel lipase powder (395 mg).

In the general procedure for lipase-catalyzed hydrolysis, the sol-gel lipase (10 μg) or free lipase (10 μl) was added to a mixture of (±)-2-octyl acetate

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1 To whom correspondence should be addressed. Fax: +81-52-736-7405; E-mail: katsuya-kato@aist.go.jp

Abbreviations: VTES, vinyltriethoxysilane; TMS, tetramethoxysilane; MTMS, methyltrimethoxysilane; PTMS, propyltrimethoxysilane

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I (5 mg) in 0.1 M phosphate buffer (pH 7, 2.7 ml) and acetone (0.3 ml). After being stirred on a reciprocal shaker at 120 rpm and at 30°C for an adequate period, the mixture was filtered, and the fluid filtrate was extracted three times with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated in vacuo. The residue was put through GLC in a chiral stationary phase column (CP-cyclodex B 236M, 0.25 mm X 30 m) under the following conditions: detector, FID; carrier gas, He; column temperature, 130°C; injection and detector temperature, 180°C. Retention times were (RS)-2-octanol (RS)-2, 11.3 min; (S)-2-octyl acetate (S)-1, 13.3 min; and (R)-2-octyl acetate (R)-1, 14.0 min.

Figure 1 shows relative hydrolysis of (+)-I by variously prepared SP 525 sol-gel lipases. The sol-gel lipase prepared from vinyltriethoxysilane (VTES) had the highest hydrolytic activity, approximately 15-20% higher than that from a mixture of propyltrimethoxysilane and tetramethoxysilane (5:1, v:v) described by Reetz et al.7) Reactivities of the sol-gel lipases prepared with alkylsiloxanes (methyltrimethoxysilane, MTMS) and tetramethoxysilane (TMS) are very low. The sol-gel material without lipase prepared by a similar procedure had no hydrolytic activity. Sol-gel preparation from VTES is very attractive because it is not an exothermic process during the NaF-catalyzed reaction. Because gelation proceeds very slowly, it is not necessary to cool the reaction mixture at 4°C to avoid a decrease of lipase activity. Furthermore, alkenyltrialkoxyxilane, which has a reactive terminal double bond, can be transformed into unique inorganic-organic materials by chemical methods.13)

We next investigated the thermal stability of sol-gel (VTES) lipase SP 525 (Fig. 2). Lipase activity was measured by the hydrolytic reaction of 2-octyl acetate at 30°C after incubation with no substrate at adequate temperatures for 1 h. This sol-gel lipase retained approximately 70% of the initial enzymatic activity after incubation at 90°C, but the remaining activity of the free lipase was only 20% after incubation at 60°C. These results suggest that surrounding the enzyme with a sol-gel matrix markedly increases the thermal stability of the lipase. Moreover, the sol-gel lipase SP 525 could be recycled at least 10 times for hydrolysis of 2-octyl acetate at 30°C, because 86% of the hydrolytic activity remained after recycling 10 times (data not shown).

Sol-gel lipases were prepared from VTES with Pseudomonas fluorescens lipase (AK, Amano, 18,500 units/g) and Ps. cepacia lipase (PS, Amano, 30,000 units/g) by a similar procedure to that
Table 1. Enantioselectivity and Reactivity for 1 with Lipases Encapsulated by the Sol-Gel Method

<table>
<thead>
<tr>
<th>Lipases</th>
<th>Time (h)</th>
<th>Conv (%)</th>
<th>Acetate (E) (%)</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol-gel SP 525</td>
<td>1</td>
<td>38</td>
<td>71</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Free SP 525</td>
<td>1</td>
<td>45</td>
<td>97</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Sol-gel AK</td>
<td>18</td>
<td>18</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Free AK</td>
<td>18</td>
<td>31</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>Sol-gel PS</td>
<td>3</td>
<td>33</td>
<td>44</td>
<td>22</td>
</tr>
<tr>
<td>Free PS</td>
<td>3</td>
<td>48</td>
<td>53</td>
<td>6</td>
</tr>
</tbody>
</table>

* Sol-gel lipase (10 mg) or free lipase (10 μl, SP 525: 0.8 mg protein/ml; AK: 0.7 mg protein/ml; PS: 0.8 mg protein/ml) was added to a solution of 1 (10 mg) and acetone (0.3 ml) in 0.1 M phosphate buffer (pH 7, 2.7 ml) at 30°C.

* Conversion rate (%) was measured by chiral GC.

* Enantiomeric ratio (E) was calculated as in reference 12.

described above. Enantioselective hydrolysis of these lipases was examined (Table 1). Both the sol-gel SP 525 and free lipases had excellent enantioselectivity for 2-octyl acetate (E > 100). Significantly, the enantioselectivity of lipase AK was increased from E = 5 to 9 and that of PS from E = 6 to 22. Although the precise mechanism resulting in these observed effects of lipase entrapment in a sol-gel matrix is not clear, it is reasonable to assume that the phenomenon is the result of a conformational change in the lipase enzyme induced by specific interaction between the lipase and sol-gel matrix.

In addition, a preparative-scale reaction of optically active 2-octanol 2 was done by sol-gel lipase-catalyzed enantioselective acylation with vinyl acetate as the acyl donor and as the solvent. The reaction proceeded smoothly, both enantiomers of 2-octanoyl acetate 1 being obtained in good optical yields. Sol-gel lipase SP 525 (100 mg) was added to a mixture of (±)-2-octanol 2 (2.0 g) and vinyl acetate (1.7 g). After being stirred vigorously at room temperature for 17 h, the mixture was filtered, and the filtrate evaporated in vacuo. The residue was purified by silica gel column chromatography (dichloromethane) giving (R)-1 [1.3 g, 47% yield, [α]D 21 +1.8 (c = 0.7, EtOH)]. Further elution with (dichloromethane/ethyl acetate = 1:1) gave (S)-2 [1.0 g, 50% yield, [α]D 21 +9.8 (c = 0.9, EtOH); standard sample (S)-2 from Aldrich, [α]D 21 +10.1 (c = 1.2, EtOH)]. A solution of (S)-2 (0.9 g) in 3 ml of acetic anhydride was stirred under reflux for 1 h, poured into ice-cold water, and then extracted three times with ethyl acetate. The organic layer was washed with water and saturated saline, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated in vacuo. The residue purified by silica-gel column chromatography with dichloromethane as the eluent, gave (S)-1 [1.0 g, 82% yield, 98% e.e., [α]D 21 +1.6 (c = 1.3, EtOH)]. IR and NMR spectra of both the enantiomers were identical with those of (±)-1.

In conclusion, lipase encapsulated in a sol-gel matrix prepared from vinyltriethoxysilane had high hydrolysis activity in aqueous solution and was thermally stable. Moreover, the enantioselectivities of lipases were increased by encapsulation in sol-gel matrices. Further investigations of the enantioselectivity and reactivity of lipases encapsulated in sol-gel matrices are in progress.

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References


