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Combination of bioimprinting and silane precursor alkyls improved the activity of sol–gel-encapsulated lipase

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ABSTRACT

Bioimprinting and sol–gel encapsulation of lipases by silane precursors are efficient methods of enhancing lipase performance in non-aqueous medium. The correlation between bioimprinting, the alkyl-chain length of silane precursors, and the catalytic activity of gel-encapsulated lipase was investigated using a series of silane precursors: methyltrimethoxysilane (MTMS), vinyltrimethoxysilane (VTMOS), vinyltriethoxysilane (VTEOS), and n-octyltrimethoxysilane (OTMOS). The optimal parameters for lipase immobilization were also determined. Both bioimprinting and increasing the chain-length of alkyl groups, apparently by increasing hydrophobicity, significantly improved the specific activity and the total activity of the immobilized lipase. Compared to a non-imprinted MTMS/TMOS gel, the specific activity of an imprinted OTMOS/TMOS gel improved 14.4-fold, and the total activity improved 6.8-fold. Nitrogen adsorption–desorption assays and gel matrix surface characterization showed that the bioimprinting molecule and the hydrophobic alkyl groups of silane triggered lipase to change from the closed to the open conformation, and contributed to creating sol–gel matrices that were more porous and with less mass transfer resistance structure, apparently improving the activity of encapsulated lipase.

1. Introduction

In non-aqueous medium, lipases (EC 3.1.1.3) exhibit novel esterification and transesterification properties that make them promising biocatalysts for fat or oil processing [1], chiral resolution [2], and preparation of pharmaceuticals [3] and biofuels [4].

Lipases are members of the α/β-hydrolase fold family. The active site of lipases consists of a Ser-His-Asp/Glu catalytic triad that is completely buried beneath a short amphiphilic helical segment, which prevents substrate access. Contact with hydrophobic substrates causes a structural rearrangement, and the enzyme changes from a closed to an open conformation that makes the active site accessible [5,6].

In an immobilizing sol–gel process, the conformational change from closed to open can also be induced by bioimprinting molecules, and by the hydrophobic alkyl groups of silane precursors, and then the open conformation compatible with such molecules is trapped in the step of gelation. Compared to non-imprinted counterparts, the reaction rate of imprinted enzymes is greatly enhanced [7,8]. Bioimprinting a sol–gel that encapsulates the lipases has been shown to be an efficient method of enhancing their performance in non-aqueous medium [9,10].

Although the effects of bioimprinting and the alkyl groups of silane on lipase activity have been reported [7–12], a systematic evaluation of their contribution to the lipase activity and the effect of gel texture on enzymatic performance is needed. Combining the two factors of bioimprinting molecules and alkyl groups in a sol–gel procedure, under optimal parameters, is expected to generate enhancing effects. To find the optimal parameters for lipase encapsulation, both the fatty acid bioimprinting molecule and the alkyl chains of the silane precursors in the synthesis process were examined. The structure and texture of the resulting gel matrices were also investigated.

2. Materials and methods

2.1. Materials

Silane methyltrimethoxysilane (MTMS, 98%), tetramethoxysilane (TMOS, >99%), vinyltrimethoxysilane (VTMOS), vinyltriethoxysilane (VTEOS), and n-octyltrimethoxysilane (OTMOS) were from Aldrich. Lauric acids were from Sigma. All other solvents and reagents were obtained commercially and were of analytical grade. Crude *Burkholderia cepacia* lipase was produced in our laboratory and protected by dextrin [13]. Lipase activity checked in aqueous phase is about 36 U/mg, and protein content determined by the Bradford method was 1%.

2.2. Enzyme encapsulation in silica and bioimprinting

Lipase solution was prepared by dissolving crude lipase powder in 10 mmol/l Tris–HCl (pH 7.5) at 1.3 mg/ml protein. In 50 ml Falcon tubes, an aqueous lipase

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Table 1
Activity of lipases immobilized in imprinted and non-imprinted sol–gel particles.

Treatment	Silane	Molar ratio	Immobilization efficiency (%)	Specific activity (μmol/h mg)	Total activity (μmol/h/g gel)
Non-imprinted	MTMS/TMOS	5:1	0.92	1074.8	1233.1
	VTMOS/TMOS	7:1	0.94	1601.4	1400.9
	VTEOS/TMOS	3:1	0.98	1970.2	1662.0
	OTMOS/TMOS	1:1	0.99	3552.8	4614.3
Imprinted	MTMS/TMOS	5:1	0.66	2058.8	2393.4
	VTMOS/TMOS	7:1	0.48	3311.0	2506.9
	VTEOS/TMOS	3:1	0.53	4069.2	3711.9
	OTMOS/TMOS	1:1	0.41	15425.4	8346.4

(about 2 mg protein) solution was added to a mixture of 100 μl aqueous sodium fluoride, 90 μl polyethylene glycol (PEG400), and 50 μl isopropanol. After shaking for 5 min the silane precursors were added into the solution to achieve a water/silane molar ration = 12:1. The two-phase mixture was vigorously shaken for 5 s on a vortex mixer and then kept on ice until gelation. The reaction vessel was left closed for 24 h, and then the gel was air-dried at 37 °C for 3 d. The white gel was ground to powder with particles diameter less than 20 μm, and then washed thoroughly with Tris–HCl (pH 7.5) for 2 h and acetone for 2 h to remove uncrosslinked silane and free enzymes, before air-drying. For bioimprinting, 0.2 mmol lauric acid was added into the sol–gel mixture, and then went to the step of gelation, washing and drying steps described above.

The immobilization efficiency was calculated as the difference between the total loaded protein and the protein in the supernatant after washing, using the equation: $I\,(\%) = (P_T - P_S)/P_T \times 100$, where I is the immobilization efficiency (%), P_T is the total protein loaded (g), and P_S is the protein in the supernatant (g).

2.3. Esterification

Water-saturated isooctane solutions were made in 10 ml of 0.2 mol/1 lauric acid, 0.2 mol/l lauryl alcohol and 50.0 mg immobilized lipases. Reaction mixtures were shaken at 200 rpm at 37 °C in a 50 ml Falcon tube. A 2.0 ml aliquot was taken at intervals and the residual fatty acid levels analyzed using KOH titration methods [14]. Esterification activity was calculated as the transformation of fatty acid to its ester per hour (μmol/h). Specific esterification activity was determined by calculating the transformation of fatty acid to ester, and defined as micromoles per hour per milligram of protein (mmol/h mg).

2.4. Specific surface area and porous diameter assays

Nitrogen adsorption–desorption assays of silane particles were conducted on a TriStar3000 (Micromeritics) according to Woo et al. [15]. Specific surface areas were determined by the Brunauer–Emmett–Teller (BET) model, and the average pore diameter was determined by the Barrett–Joyner–Halenda (BJH) method [16].

2.5. Field emission-scanning electron microscope (FESEM) assays

Sample preparation, observation and data recording were conducted according to standard procedures [17]. The surface structure of the gels was observed by FESEM (Sirion 200, FEI Ltd., Holland).

3. Results and discussion

3.1. Influence of bioimprinting and silane precursors on lipase activity

According to our previous investigation [10], lauric acid was selected as a bioimprinting molecule in this study. The specific esterification activity (μmol/h mg protein), immobilization efficiency (%) and total activity (μmol/h g gel) of the immobilized enzyme were determined. As shown in Table 1, the optimal molar ratios of the precursors were 5:1 for MTMS/TMOS, 7:1 for VTMOS/TMOS, 3:1 for VTEOS/TMOS and 1:1 for OTMOS/TMOS. Specific activity and total activity of immobilized enzymes increased significantly with an increase in alkyl-chain length. The activity of immobilized enzymes prepared in OTMOS/TMOS was 4614.3 μmol/h g gel, 3.7-fold higher than that obtained with MTMS/TMOS (1233.1 μmol/h/g gel).

Compared to non-imprinted enzymes, the specific activity of the lauric acid-imprinted lipase improved significantly by 1.92-fold for MTMS/TMOS, 2.07-fold for both VTMOS/TMOS and for VTEOS/TMOS, and 4.34-fold for OTMOS/TMOS. Total activity improved 1.94-, 1.79-, 2.23- and 1.81-fold, respectively, although immobilization efficiency decreased correspondingly. Compared to non-imprinted MTMS/TMOS gel, the specific activity for OTMOS/TMOS was improved by 14.4-fold and total activity improved 6.8-fold.

3.2. Surface area, pore volume and pore diameter of gel matrices

To investigate the effect of bioimprinting and alkyl groups on the gel matrix structure, the surface area, pore volume and pore diameter were evaluated by nitrogen adsorption–desorption methods (Table 2 and Fig. 1). Silane precursors significantly influenced the properties of the gel matrices. Comparing MTMS to OTMOS, the average pore diameter of both non-imprinted and imprinted gels increased gradually, and comparing VTMOS to OTMOS, the surface area and gel pore volume also significantly improved.

After bioimprinting, the pore volume showed no change. The average pore width of the imprinted gel appeared to be significantly increased compared to the non-imprinted gel, and this coincided with an increase in lipase activity (Table 2). Wide pores might facilitate entry of the substrate into the gel matrix and thereby enhance the reaction velocity. As seen in Fig. 1, the gel prepared with OTMOS/TMOS had evenly distributed pores with diameters in the range of 8-20 nm, indicated that this gel matrix was the best of those tested in this study.

Although the gel prepared with MTMS/TMOS had a high surface area, the detected lipase activity was very low, which appeared

Table 2
Pore characteristics of silica gels with different additives.

Treatment	Silane	BET surface Area (m^2/g)	Pore volume (cm^3/g)	Average pore width (nm)
Non-imprinted	MTMS/TMOS	61.46	0.043	2.78
	VTMOS/TMOS	1.16	0.003	11.65
	VTEOS/TMOS	5.52	0.018	13.04
	OTMOS/TMOS	54.52	0.213	15.64
Imprinted	MTMS/TMOS	38.15	0.029	3.04
	VTMOS/TMOS	1.02	0.004	14.42
	VTEOS/TMOS	13.03	0.030	16.17
	OTMOS/TMOS	42.03	0.211	20.10