STUDIA UBB CHEMIA, LIX, 4, 2014 (p. 33-38)
(RECOMMENDED CITATION)

SURFACE MODIFICATION OF SILICA GELS FOR SELECTIVE ADSORPTION OF BACTERIAL LIPASES

ZOLTÁN BOROS[a,b], EMESE ABAHÁZIOVÁ[a], DIÁNA WEISER[a], PÉTER KOVÁCS[c], CSABA PAIZS[d], LÁSZLÓ POPPE[a,b,*]

ABSTRACT. Since immobilization of lipases enhances their productivity, stability and selectivity, a series of surface modified silica gel supports was developed and used for hydrophobic adsorption of Lipase AK from *Pseudomonas fluorescens* and Lipase PS from *Burkholderia cepacia.*

Keywords: *silica gel, surface modification, adsorption, lipase, immobilization, Pseudomonas fluorescens, Burkholderia cepacia*

INTRODUCTION

The use of enzymes as biocatalysts has acquired ever increasing importance in organic chemistry. The native enzymes are, however, expensive, relatively unstable and difficult to handle. Being water soluble, removal of the enzyme or its degradation products from the product may be cumbersome and the recovered enzyme usually cannot be reused. Immobilization of enzymes is an established technique and several of such biocatalysts are commercially available and applied at industrial scale.[1,2,3] The importance of this field is emphasized by a recent exhaustive review on the application of immobilized lipases in reactions conducted in organic solvents[4] as well as an in depth study of the molecular mechanism of acylation with immobilized lipases on derivatized silica gels.[5]

Immobilization of enzymes has many advantages. Being solids, they can be easily recovered and, after the reaction they can often be reused.[6] The

[a] *Budapest University of Technology and Economics, Department of Organic Chemistry and Technology, Műegyetem rkp. 3., H-1111 Budapest, Hungary*
[b] *SynBiocat Ltd., Lázár deák u. 4/1., H-1173 Budapest, Hungary*
[c] *Research Centre for Natural Sciences, Institute of Organic Chemistry, Hungarian Academy of Sciences, Magyar tudósok körútja 2., H-1117 Budapest, Hungary*
[d] *Biocatalysis and Biotransformation Research Group, Babes-Bolyai University of Cluj-Napoca, Arany János str. 11, Ro-400028 Cluj-Napoca, Romania*
[*] *Corresponding author: poppe@mail.bme.hu*

catalytic properties of immobilized enzymes, such as stability, activity and selectivity, can be efficiently influenced by the proper choice of the solid support.

A further advantage of immobilized enzymes is that they can be used in syntheses similar to conventional biocatalysts. A disadvantage of the latter is that, since they operate in homogeneous solutions, the enzyme, or its degradation products, may appear as contaminants in the product the removal of which may be difficult. Immobilization avoids these problems and, as a further advantage, they can be used in continuous-flow reactors.[7]

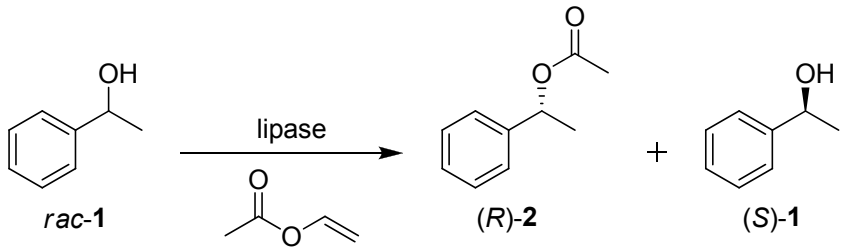
Lipases (triacylglycerol esterases EC 3.1.1.3) catalyzing the hydrolysis of lipids can be found basically in every living organism and their interfacial activation occurs at specific lipid-water interfaces.[8] Biocatalysts ensure a clean and environmentally friendly way to carry out chemical reactions under mild conditions with high stereoselectivity.[9] Therefore, the use of enzymes, especially in organic solvents, 4has a great potential in the manufacturing of a single enantiomer of chiral drugs.[10] As a result, biotransformations are therefore nowadays a generally accepted method for the synthesis of such drugs.[11]

The objective of the present study was to develop, by surface modifications, silica gel supports which would ensure high efficiency, enantioselectivity and stability of the lipase attached to them.

RESULTS AND DISCUSSION

First, surface modification was carried out by derivatization with mono- and disubstituted alkoxysilanes. The silica gels thus modified were used as carriers in adsorptive immobilization of Lipase AK (from *Pseudomonas fluorescens*) and Lipase PS(from *Burkholderia cepacia*).

Next, the hydrophobic adsorption of Lipase AK was carried out onto 19 surface modified silica gel supports. The modified biocatalysts were tested in a model reaction, in the enantioselective acylation of racemic 1-phenylethanol rac-1 with vinyl acetate (Scheme 1).



Scheme 1. *Kinetic resolution screen for the immobilized bacterial lipases*

Table 1. Biocatalytic properties of Lipase AK adsorbed on various surface-modified silica gels tested by kinetic resolution of rac-**1** in n-hexane:MTBE 2:1 at 4 h.

Silicagel derivatization	c [%][a]	$ee_{(R)\text{-}2}$[%][a]	E[b]	U_B[c] [μmol min^{-1} g^{-1}]
-	1.1	98.6	146	0.4
Methyl	27.5	99.4	467	19.0
Ethyl	19.7	99.4	410	13.6
Propyl	17.0	99.4	426	11.8
Isobutyl	9.0	99.4	367	6.2
Hexyl	12.2	99.4	387	8.4
Octyl	38.3	99.2	440	26.4
Decyl	15.5	99.4	393	10.7
Dodecyl	16.6	99.4	392	11.5
Octadecyl	24.0	99.5	521	16.6
Phenyl	13.4	99.6	553	9.3
Perfluorooctyl	26.9	99.5	528	18.5
Vinyl	22.9	99.3	375	15.8
2-Cyanoethyl	14.3	99.6	600	9.9
3-Chloropropyl	24.9	99.5	506	17.2
3-Mercaptopropyl	24.0	99.5	516	16.6
3-Amino-2-hydroxypropyl	6.2	99.9	1380	4.3
Dimethyl	16.4	99.5	441	11.3
Phenyl/methyl	13.4	99.6	553	9.3
Cyclohexyl/methyl	16.1	99.6	552	11.1

[a] Conversion (c) and enantiomeric excess (ee) were measured by GC.[12]
[b] Enantiomer selectivity (E) was calculated from c and $ee_{(R)\text{-}2}$.
[c] Specific biocatalyst activities (U_B) were calculated by the equation $U_B = n_P / (t \times m_B)$ (where n_P [μmol] is the amount of the product, t [min] the reaction time and m_B [g] the mass of the applied biocatalyst).

Results after 4 hours reaction time are shown in Table 1. Enantioselectivity was high (>99%) for all variants. The highest activity (U_B) was achieved with octyl, methyl, perfluorooctyl and 3-chloropropyl grafted silica supports among which the perfluorooctyl variant was the most selective. Lowest activity was displayed by the enzymes immobilized onto isobutyl- and 3-amino-2-hydroxypropyl grafted silica supports.

After experiments with Lipase AK, the adsorption of Lipase PS was carried out. Results after 4 hours reaction time are shown in Table 2.

As shown above, adsorption of Lipase AK to the perfluorooctyl-grafted silica gave a highly selective biocatalyst (ee=99.5%). Adsorption of Lipase PS on the same support gave even better enantioselectivity (ee=99.6%). With the dodecyl and octadecyl grafted variants, both activity (c=6.5 and6.2%)

and selectivity (*ee*=99.3% and98.5%) were low. Selectivity of the octadecyl grafted support was below 99% i.e. it was of the worst selectivity. The most productive carriers were 3-chloropropyl, 2-cyanoethyl, phenyl and phenyl-methyl grafted supports (*c*=38,8%, 33,9%, 33,6%, and32,9% respectively). These supports showed high enantioselectivity as well. Enantioselectivity was the highest with the hexyl grafted variant (*ee*=99.9%). In general, it was established that the nature of surface modification of the support significantly improved the biocatalytic potential of the adsorbed enzymes. It is of note, that Lipase PS immobilized onto an unmodified support had the lowest activity (*c*=3.2%) and almost all of those attached to a modified carrier proved to be more enantioselective. From Table 2 it can be concluded that the optimal carriers are those grafted with3-chloropropyl, 2-cyanoethyl and phenyl groups.

Table 2. Biocatalytic properties of Lipase PS adsorbed on various surface-modified silica gels tested by kinetic resolution of rac-**1** in n-hexane:MTBE 2:1 at 4 h.

Silica gel derivatization	c [%][a]	$ee_{(R)\text{-}2}$[%][a]	E[b]	U_B[c] [μmol min^{-1} g^{-1}]
-	3.2	99.8	886	2.2
Methyl	31.1	99.6	767	21.6
Ethyl	24.9	99.7	857	17.1
Propyl	27.7	99.7	984	19.1
Isobutyl	8.0	99.3	317	5.5
Hexyl	10.4	99.9	2257	7.2
Octyl	28.2	99.7	970	19.4
Decyl	12.8	99.7	653	8.9
Dodecyl	6.5	99.3	288	4.5
Octadecyl	6.2	98.5	138	4.3
Phenyl	33.6	99.7	1088	23.1
Perfluorooctyl	22.9	99.6	729	15.9
Vinyl	30.9	99.6	855	21.4
2-Cyanoethyl	33.9	99.6	867	23.5
3-Chloropropyl	38.8	99.7	1101	26.9
3-Mercaptopropyl	27.6	99.7	883	19.0
3-Amino-2-hydroxypropyl	16.1	99.7	688	11.2
Dimethyl	29.7	99.7	998	20.6
Phenyl/methyl	32.9	99.7	1178	22.7
Cyclohexyl/methyl	27.8	99.7	1029	19.3

[a] Conversion (*c*) and enantiomeric excess (*ee*) were measured by GC.[12]

[b] Enantiomer selectivity (*E*) was calculated from *c* and $ee_{(R)\text{-}2}$.

[c] Specific biocatalyst activities (U_B) were calculated by the equation $U_B = n_P / (t \times m_B)$ (where n_P [μmol] is the amount of the product, t [min] the reaction time and m_B [g] the mass of the applied biocatalyst).

CONCLUSIONS

For the adsorption of Lipase AK, the best carriers were octyl, methyl and perfluorooctyl grafted silica gels, while for the adsorption of Lipase PS, 3-chloropropyl, phenyl and 2-cyanoethyl functionalizations were the most appropriate. Our results demonstrated that among the modified silica gels tested in the present study there cannot be found a support which simultaneously exhibits optimum selectivity and activity. Adsorption is a two-way physical process that depends on the nature of both the enzyme and its support.

EXPERIMENTAL SECTION

Chemicals and enzymes

Racemic 1-phenylethanol, vinyl acetate and all further chemicals and solvents were of analytical grade or higher and were purchased from Sigma-Aldrich (St. Luis, MO, USA) or Merck(Darmstadt, Germany). Lipase PS and AK were the products of AmanoEnzyme (Nagoya, Japan). Surface functionalized silica gels were the products of SynBiocat (Budapest, Hungary).

Analytical methods

GC analyses were carried out on an 4890 instrument, Agilent (Santa Clara, CA, USA) equipped with a FID detector and a Hydrodex β-6TBDM column [25 m × 0.25 mm × 0.25 μm film with heptakis-(2,3-di-*O*-methyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodextrine; Macherey & Nagel (Düren, Germany)] using H_2 as carrier gas (injector: 250°C, FID detector: 250°C, head pressure: 12 psi, 50:1 split ratio). GC data (oven program), t_r (min): for rac-1 and rac-**2** (120°C, 8 min), 4.0 [(*S*)-**2**], 4.4 [(*R*)-**2**], 5.8 [(*R*)-**1**], 6.0 [(*S*)-**1**].

Adsorption of enzymes on surface modified silica gels

Enzymes were dissolved in Tr is buffer (11.25 mL, 100 mM, pH=7.5, ionic strength controlled with NaCl) then the surface functionalized silica gel (250 mg) was added. The mixture was incubated at 400 rpm and 4°C for 18 h. The immobilized enzymes were filtered off on a glass filter (G4), washed with 2-propanol (2x5 mL), hexane (5 mL), dried at room temperature (2 h) and stored at 4°C.

Enantiomer selective acetylation of racemic 1-phenylethanol *rac*-1 in shake vials

To a solution of racemic 1-phenylethanol *rac*-**1** (101 mg; 0.828 mmol) in a mixture of hexane, *tert*-butyl-methyl-ether and vinyl acetate 6/3/1 (2 mL), the enzyme (50 mg) was added. The mixture was shaken (1000 rpm) in a sealed amber glass vial at 30°C for 4 hours. The products were analyzed by GC and TLC after 1, 2, and 4 hours.

ACKNOWLEDGMENTS

This research was part of the scientific program "*Talent care and cultivation in the scientific workshops of BME" (TÁMOP-4.2.2.B-10/1–2010–0009*), supported by the New Hungary Development Plan.

REFERENCES

1. Umemura, S.; Takamatsu, T.; Sato, T.; Tosa, I.; Chibata, I., *Appl. Microbiol. Biotechnol.*, **1984**, *20*, 291-295.
2. Kennedy, J.F.; Melo, E.H.M.; Junel,K., *Chem. Eng. Progress*, **1990**, *7*, 81-89.
3. Parathasarathy, R.V.; Martin, C.R., *Nature*, **1994**, *369*, 298-301.
4. Adlerkreuz, P.; *Chem. Soc. Rev.,* **2013***, 42(15)*, 6406-6436.
5. Jiu, D.; Jia, G.; Zhang, Y.; Yang,Q.; Li, C., *Langmuir*, **2011**, *27*, 12016-12024.
6. Cao L. "*Carrier-bound Immobilized Enzymes: Principles, Application and Design*" Wiley-VCH, Wienheim, **2005**.
7. Itabaiana, I. Jr.; de Mariz e Miranda, L.S.; de Souza, R.O.M.A.,*J. Mol. Catal. B Enzym.*, **2013**, *85-86*, 1-9.
8. Reetz, M.T., *Curr. Opin. Chem. Biol.*, **2002**, *6*, 145-150.
9. Gotor-Fernández, V.; Brieva, R.; Gotor, V., *J. Mol. Catal. B Enzym.*, **2006**, *40*, 111-120.
10. Margolin, A.L., *Enzyme Microb. Technol.*, **2003**,*15*, 266-280.
11. Patel, R.N., *Curr. Opin. Drug Discov. Dev.*, **2003**,*6*, 902-920.
12. Chen, C.S.; Fujimoto, Y.; Girdaukas, G.; Sih, C.J., *J. Am. Chem. Soc.*, **1982**, *104*, 7294-7299.