In situ fixation of lipase-containing hydrophobic sol–gel materials on sintered glass—highly efficient heterogeneous biocatalysts

Manfred T. Reetz,* Albin Zonta, Jörg Simpelkamp and Werner Könen

Max-Planck-Institut für Kohlenforschung, Kaiser-Wilhelm-Platz 1, D-45470 Mülheim/Ruhr, Germany

The previously reported process of entrapping lipases in hydrophobic organic/inorganic hybride silica gels is extended in that the entrapment by the sol–gel process is performed in the presence of porous glass beads, *e.g.* (SIRAN®), resulting in the *in situ* fixation of the sol–gel material on the surface of the support and formation of highly active and mechanically stable heterogeneous biocatalysts.

Recently we described the entrapment of lipases in hydrophobic sol–gel materials with formation of heterogeneous biocatalysts having significantly enhanced enzyme activities.[1] Accordingly, aqueous solutions of lipases are treated with lipophilic silanes of the type $RSi(OMe)_3$ in a sol–gel process,[2] affording alkyl-modified silica gels containing the enzymes. Mixtures of $RSi(OMe)_3$ and $Si(OMe)_4$ or $RSi(OMe)_3$ and polydimethylsiloxane can also be used successfully, but not $Si(OMe)_4$ alone.[1,3] Since lipases are surface active enzymes,[4] the hypothesis that lipophilic domains of these catalysts interact with the alkyl groups of the silica matrix was advanced.[1] Increased enzyme activities of up to 8800% (factor of 88) relative to the use of traditional suspensions of the lipase[5] in organic solvents were observed, as shown by kinetic studies of the model reaction involving the esterification of lauric acid by octan-1-ol.[1] We now report a significant advancement in this field,[6] namely the specific fixation of the previously described lipase-containing organic/inorganic hybrid matrices[1] on sintered glass, resulting in heterogeneous biocatalysts of even higher activity, ready re-usability and increased mechanical stability.

Our strategy involves the hydrolysis of the silanes in the presence of a lipase and some form of sintered glass such as SIRAN®.[7] The latter is a coarse glass bead with a relatively low surface area (*ca.* 0.2 $m^2\ g^{-1}$),[8] a material which has been used to immobilize microorganisms[9] and mammalian cells.[10] Before performing the actual enzyme immobilization, exploratory experiments with silanes and SIRAN® in the absence of a lipase were carried out.[11] In doing so, we discovered that there is an optimal ratio of silane to SIRAN®. For example, if not enough SIRAN® is used, a good portion of the solid product is simply the normal fine-grained sol–gel material. In contrast, if too much SIRAN® is present, all of the sol–gel material becomes attached to the outer surface of the bead, but some of the latter is not covered completely.

Upon mixing the gel-precursors, *e.g.* TMOS/PDMS,† an aqueous solution of lipase SP 523, polyvinyl alcohol as an additive[3] and catalytic amounts of NaF, the proper amount of SIRAN® was added.‡ The sol–gel process with concomitant entrapment of the lipase proceeded in such a way that >95% of the sol–gel material selectively became attached to the surface of the beads. Similar results were obtained upon using other combinations of silanes and SIRAN®, although lipase-entrapment is not always complete, Table 1. As summarized in Table 1, these materials show significantly enhanced enzyme activities in the catalysis of the model esterification reaction relative to the use of traditional suspension of the enzyme under otherwise identical conditions. Relative to the sol–gel materials described previously,[1] the present heterogeneous biocatalysts are, in optimal cases, more active by one order of magnitude. In a control experiment the lipase SP 523 was also immobilized by conventional adsorption on SIRAN®. Although a relatively high corrected activity was observed, Table 1, only 12% of the enzyme could be adsorbed which means that the uncorrected relative activity is low. Re-usability also differs dramatically.

In order to characterize the catalysts, samples of SIRAN® before and after the sol–gel process were examined by scanning electron microscopy (SEM). Fig. 1(*a*) contains the resulting micrograph of SIRAN® alone, visualizing the porous nature of the material. Fig. 1(*b*) shows the same glassy bead following treatment by the sol–gel process. It is clear that the hollow spaces ('caves') in the beads have been filled. Interestingly, magnification of the micrograph, *i.e.* the close-up of the lipase-containing sol–gel 'filling' shows an amorphous, porous structure Fig. 1(*c*). Thus, this morphology is different from that of the same sol–gel material obtained in the absence of SIRAN®. In the latter case small spherical particles are generally observed.[1,3] The cracks visible in Fig. 1(*b*) are probably due to shrinkage during the drying process, and may actually have a positive effect on enzyme accessibility and therefore overall catalytic activity.

The present heterogeneous catalysts are chemically and mechanically very stable. In contrast to the material obtained by simple adsorption of the lipase on SIRAN®, which loses >80% of its activity after several reaction runs, the sol–gel/SIRAN® samples can be re-used many times without marked loss of enzyme activity (<15%). The mechanical stability is significantly higher than that of the previously described lipase-containing sol–gel catalysts.[1] The controlled attachment of the sol–gel material to the SIRAN®- surface is most likely covalent *via* Si–O–Si bridges. Indeed, the free OH-groups at the surface of the glass probably provide a nucleation point for the sol–gel condensation process. The heterogeneous biocatalysts can be recycled by simple decantation and washing. In the case of commercial lipase powders as catalysts in traditional esterification reactions,[5] this is generally not possible because the water which is formed during the reaction leads to a viscous enzyme-containing residue which is difficult to separate.[1]

Table 1 Immobilizates of lipase SP 523 using SIRAN® and their activities in the esterification of lauric acid by octanol in isooctane

Immobilizate Gel precursors (molar ratio)	Spec. activity/ μmol $h^{-1}\ mg^{-1}$	Degree of immobilization (%)	Relative activity (%)[a]
MTMS/PDMS(6:1)[b]	143.9	73	157 (217)
TMOS/PDMS(4:1)[b]	165.7	100	180 (180)
TMOS/PTMS(1:5)[b]	172.9	79	188 (239)
SMS/PDMS(0.9:1)[b]	141.5	96	154 (161)
Lipase SP 523[c]	18.9	12	21 (172)
commercial ('free') lipase SP 523 powder	0.92	—	— (—)

[a] Relative activity with respect to that of non-immobilized lipase SP 523 powder (corrected values in brackets). In calculating the corrected relative activity, the degree of immobilization is considered. [b] *In situ* fixated on SIRAN®. [c] Adsorbed on SIRAN®.

The presented biocatalysts are ideal materials for fluid-bed reactors. For example, continuous interesterification of triolein with lauric acid in water-saturated hexane catalysed by sol–gel immobilized lipase PS (Amano) on SIRAN® showed very high operational stability. An equimolar mixture of glyceryl trioleate (triolein) and lauric acid (0.1 mol dm^{-3}) was dissolved in water-saturated hexane and continuously pumped through a column packed with sol–gel coated SIRAN® beads containing entrapped lipase PS (flow rate 10 ml h^{-1}, 11.8 g catalyst). Samples were taken periodically for conversion analysis by GC. The residual activity of the catalyst after 3 months is about 63%. This surprisingly high value clearly indicates that the sol–gel coated glass beads can be used as efficient catalyst for derivatization and raffination of fats and oils under long term conditions.

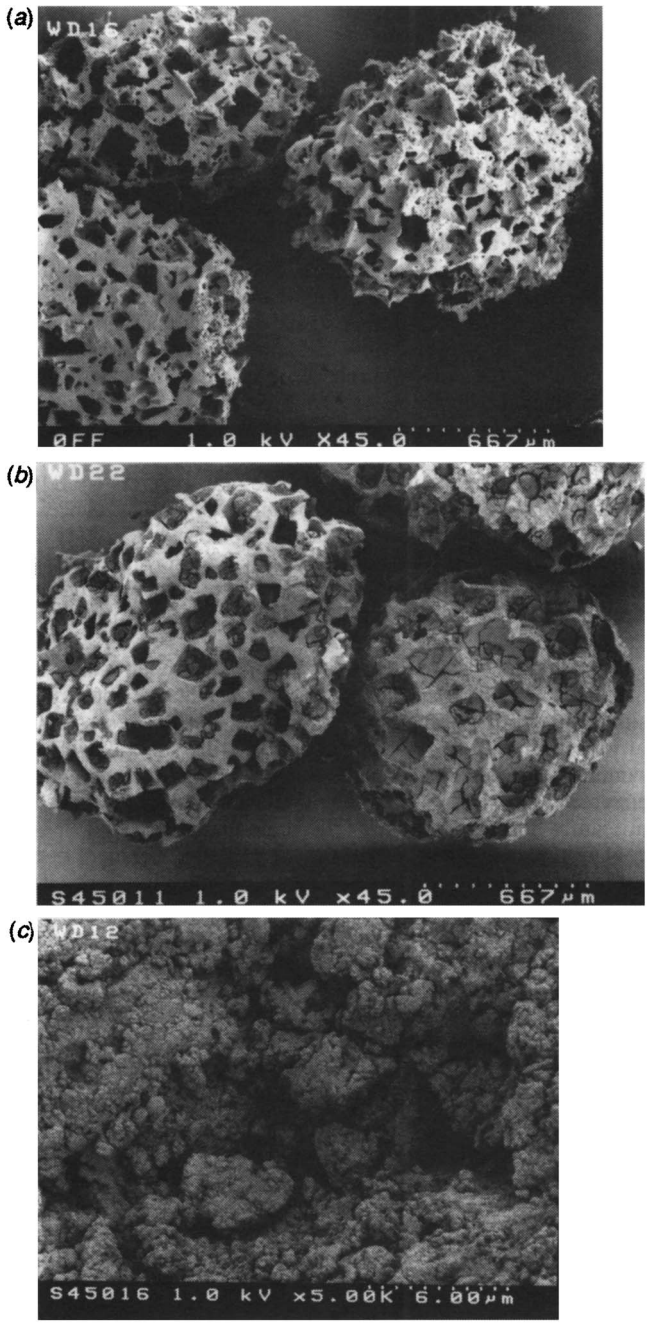

Fig. 1 (*a*) SEM-visualization of untreated SIRAN®; (*b*) SEM-visualization of a lipase SP 523 containing TMOS/PDMS (4 : 1) gel on SIRAN®; (*c*) *ca.* 100-fold magnification of SEM-picture in (*b*)

In summary, the entrapment of lipases in hydrophobic organic/inorganic hybride silica gels with complete concomitant attachment to the outer surface of porous glass beads affords highly active and mechanically stable heterogeneous biocatalysts which are useful in batch or fluid-bed reactors. The idea of entrapping biocatalysts in sol–gel materials,[1,12] with simultaneous fixation thereof on porous glass beads may also be of interest in the case of traditional metal catalysts.

We thank Novo Nordisk A S (Denmark) for a sample of Lipase SP 523 and Amano Enzyme Europe Ltd. (England) for Lipase PS (Amano).

Footnotes

† Abbreviations: Methyltrimethoxysilane (MTMS), Tetramethoxysilane (TMOS), Polydimethylsiloxane (PDMS), Propyltrimethoxysilane (PTMS), Sodiummethylsiliconate (SMS).

‡ Typical immobilization: To a mixture of 100 µl buffered solution of lipase SP 523 in 0.1 mol dm^{-3} sodium phosphate (pH 7.0), 100 µl polyvinyl alcohol (M_W 15000, 4% *w/w* in water) and 14 µl 1 mol dm^{-3} sodium fluoride, 217 µl PDMS (silanol-terminated, M_W 400–700) and 321 µl MTMS were added.[1] After thoroughly mixing the liquid components 1 g SIRAN® beads were added so that no liquid supernatant was left. The mixture was mixtured thoroughly again until gelation occurred (*ca.* 2 min). The coated glass beads thus obtained were allowed to stand in a sealed vessel for 24 h, air-dried for 3 d at 37 °C and washed with water, acetone and pentane.

References

1 M. T. Reetz, A. Zonta and J. Simpelkamp, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 301; M. T. Reetz, A. Zonta and J. Simpelkamp, *Biotechnol. Bioengo.*, 1996, **49**, 527.
2 L. L. Hench and J. K. West, *Chem. Rev.*, 1990, **90**, 33; C. J. Brinker and W. Scherer, *Sol–Gel-Science: The Physics and Chemistry of Sol–Gel-Processing*, Academic Press, Boston 1990.
3 M. T. Reetz, J. Simpelkamp and A. Zonta, Ger. Pat. Application P 4 408 152.9.
4 A. M. Brzozowski, U. Derewenda, Z. S. Derewenda, G. G. Dodson, P. W. Lawson, J. P. Turkenburg, F. Bjorkling, B. Huge-Jensen, S. A. Patkar and L. Thim, *Nature*, 1991, **351**, 491; K.-E. Jaeger, S. Ransac, B. W. Dijkstra, C. Colson, M. van Heuvel and O. Misset, *FEMS Microbiol. Rev.*, 1994, **15**, 29.
5 K. Faber, *Biotransformations in Organic Chemistry*, Springer- Verlag, Berlin 1995; *Enzymes as Catalysts in Organic Synthesis*, ed. M. P. Schneider, Reidel, Dordrecht, 1986; W. Boland, C. Frößl and M. Lorenz, *Synthesis*, 1991, 1049.
6 Other methods of lipase-immobilization: F. X. Malcata, H. R. Reyes, H. S. Garcia, C. G. Hill Jr. and C. H. Amundson, *J. Am. Oil Chem. Soc.*, 1990, **67**, 890.
7 Product information sheet No. 60038 d, Schott Engineering GmbH, Germany.
8 C. Dreisbach, Diplomarbeit, Universität Bonn, 1990.
9 A. Aivasidis, C. Wandrey, H. G. Eils and M. Katzke, *Proceedings EBC Congress*, 1991.
10 E. Lüllau, C. Dreisbach, A. Grogg, M. Biselli and C. Wandrey, *Proceedings 11th ESACT Meeting*, 1991.
11 A. Zonta, Dissertation, Universität Bochum, 1995.
12 D. Avnir, *Acc. Chem. Res.*, 1995, **28**, 328.

Received, 11th March 1996; Com. 6/01688J