Journal of Materials Chemistry

Cite this: DOI: 10.1039/c2jm30759f

www.rsc.org/materials

PAPER

Paper-immobilized enzyme as a green microstructured catalyst†

Hirotaka Koga,*[a] Takuya Kitaoka[bc] and Akira Isogai[a]

Received 8th February 2012, Accepted 3rd April 2012
DOI: 10.1039/c2jm30759f

The facile and direct introduction of methacryloxy groups into cellulose paper was carried out using a silane coupling technique, leading to the improvement of hydrophobicity and both dry and wet physical strengths of the paper. Immobilization of lipase enzymes onto the methacrylate-modified paper was then accomplished, possibly due to hydrophobic interaction. The as-prepared immobilized lipase on methacrylate-modified paper possessed paper-specific practical utility. During a batch process for the nonaqueous transesterification between 1-phenylethanol and vinyl acetate to produce 1-phenylethylacetate, the paper-immobilized lipase showed high catalytic activity, selectivity and reusability, suggesting that the methacryloxy groups introduced into the cellulose paper played a key role in the hyperactivation of lipases. In addition, a higher productivity of 1-phenylethylacetate was achieved in a continuous flow reaction system than in the batch system, indicating that the interconnected porous microstructure of the paper provided favorable flow paths for the reactant solution. Thus, the paper-immobilized enzyme is expected to offer a green catalytic material for the effective production of useful chemicals.

Introduction

Enzymes have received increasing interest as green catalysts for a wide range of chemical industries because of their high catalytic activity and selectivity under mild conditions.[1,2] Amongst the many enzymes in existence, lipases (glycerol ester hydrolases E.C.3.1.1.3) are known to catalyze the hydrolysis of fats in their natural environment. In recent years, lipase-catalyzed reactions in nonaqueous media, such as transesterification, esterification, aminolysis, acyl exchange and thiotransesterification,[3] have been extensively studied for synthesis of many useful chemicals for food, cosmetic, pharmaceutical and biodiesel applications.[4–6] However, enzymes, including lipases, are frequently unstable in nonaqueous media and easily aggregate and deactivate.[7] To solve this problem, enzymes are often immobilized on various supporting materials, such as silica,[8,9] ceramics,[10,11] carbonaceous materials,[12] polymers and resins.[13,14] Such immobilized enzymes provide reusability as well as stability in nonaqueous media[15,16] and offer advantages in the isolation of products.[16]

Immobilized enzymes have been prepared through various interactions, such as physical adsorption, hydrophobic interaction, covalent and electrovalent bonding.[16] Because lipases have relatively high hydrophobicity, simple adsorption of lipases on suitably hydrophobic supports is regarded as one of the most effective approaches.[15,17–20] The hydrophobic surface of supports leads to hyperactivation of lipases *via* interfacial adsorption,[17,18] because the change in conformation of lipases caused by hydrophobic interaction provides efficient accessibility of substrates to their active centers. For this reason, the surface modification of supporting materials has been conducted to obtain suitable hydrophobicity, and ceramic supports modified with methacryloxy groups have been reported as effective supporting materials for lipases.[10,11] In addition, the structural design of porous supports has been intensely studied to enable their use as microstructured flow reactors to efficiently and continuously obtain target products.[11,21–29] However, there is still a requirement to develop high-performance immobilized enzymes with excellent practical utility.

Cellulose (β-1,4-D-glucopyranose polymer), the principal ingredient in plant-based biomass, is the most common and abundant organic polymer.[30] From the viewpoint of sustainable development, the active use of cellulosic resources is of growing importance, and many researchers have energetically developed new cellulose-based materials with various functions.[31–41] Paper is one of the most common cellulosic products, traditionally used in our daily life for various purposes such as writing, printing and packaging. Paper materials are low cost, lightweight, flexible and easy to handle, and thus have excellent practical utility. In addition, several of our previous studies indicate that the

[a]*Department of Biomaterials Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. E-mail: ahkoga@mail.ecc.u-tokyo.ac.jp; Fax: +81-3-5841-5271; Tel: +81-3-5841-5271*
[b]*Department of Agro-environmental Sciences, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan*
[c]*Biotron Application Center, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan*
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2jm30759f

paper-specific interconnected porous microstructure derived from fiber networks can provide a favorable reaction environment, especially in flow-type catalytic reactions, allowing for the effective diffusion of heat and reactants.[42–45] Therefore, paper is expected to serve both as a versatile support material and as an effective microstructured flow reactor for various catalysts, including enzymes.

We have recently reported the direct introduction of amino groups into cellulose paper using a silane coupling technique through the condensation reaction between the Si–OH of the organofunctional silane coupling agent and the C–OH of cellulose, which we refer to as the *in situ* modification of cellulose paper with amino groups.[37] The as-prepared paper material, denoted amine-modified paper, had excellent handling convenience and demonstrated high catalytic efficiency and reusability in the Knoevenagel condensation reaction. The silane coupling technique has great potential for introducing an array of functional groups into cellulose paper and to develop newly functionalized paper materials. The chemically modified cellulose paper is expected to offer a promising supporting material for enzymes.

In this study, the *in situ* modification of cellulose paper with methacryloxy groups as binding sites for lipases was investigated. The physical properties, *i.e.* mechanical strength and hydrophobicity, of the as-prepared methacrylate-modified paper were evaluated. The immobilization of lipase on methacrylate-modified paper was conducted and the catalytic performance of the immobilized lipase on methacrylate-modified paper was then investigated for a nonaqueous transesterification reaction in both batch and flow modes.

Experimental

Materials

Cellulose filter paper (ADVANTEC No. 5A, cotton linter cellulose content >99 wt%) was purchased from Toyo Roshi Kaisha, Ltd., Japan, and was cut into circular pieces (*ca.* 90 mg, 33 mm in diameter) before use. 3-(Trimethoxysilyl)propyl methacrylate (>98.0%, Tokyo Chemical Industry Co., Ltd., Japan, see ESI, Fig. S1†) was used as a silane coupling agent. Lipase from *Burkholderia cepacia* (Amano Lipase PS, lipase protein content *ca.* 5.6 μg mg^{-1} (ref. 13 and 46)) was obtained from Sigma-Aldrich Co. Ltd. (St Louis, MO, USA). (*R*,*S*)-1-Phenylethanol (>98.0%), (*R*)-1-phenylethanol (>98.0%), (*S*)-1-phenylethanol (>98.0%), vinyl acetate monomer (>99.0%), isopropyl ether (>99.0%) and 4-nitrophenyl acetate (>98.0%) were obtained from Tokyo Chemical Industry Co., Ltd., Japan. All reagents were used without further purification.

Preparation of methacrylate-modified paper by the silane coupling technique

3-(Trimethoxysilyl)propyl methacrylate (100 mg) was added to an acetic acid aqueous solution (pH = *ca.* 4, 10 ml), and the mixture was vigorously stirred for 30 min. Then it was hydrolyzed to form reactive silanol groups and thus was dissolved in the solution. Subsequently, a piece of cellulose paper was immersed in the resulting solution for 2 h, followed by evaporation of an acetic acid aqueous solution at 40 °C for 3 h under reduced pressure. The obtained paper was thermally treated at 110 °C for 3 h, then thoroughly washed with ethanol and dried at room temperature.

Immobilization of lipase on methacrylate-modified paper

Immobilization of lipase on original (unmodified) or methacrylate-modified cellulose paper was performed as follows. Crude lipase powders (10 mg) were suspended in phosphate buffer solution (pH = 6.86, ionic strength: 0.1 M, 10 ml) and a piece of original or methacrylate-modified cellulose paper was then immersed in the lipase solution with insoluble components, followed by stirring (150 rpm) by using an orbital platform shaker (Rotamax 120, Heidolph Instruments GmbH & Co. KG) at 23 °C for 12 h. The treated paper was then removed from the solution, thoroughly washed with phosphate buffer solution (pH = 6.86, ionic strength: 0.1 M, 10 ml) at a stirring rate of 150 rpm for 3 h to remove unwanted insoluble components adsorbed onto the paper, and dried in a desiccator at room temperature. The lipase content in the as-prepared paper was measured in part according to the method described by previous reports:[25,47] enzymatic activity of the lipase solutions (supernatant, not paper) before and after soaking treatment of the paper was determined using a colorimetric assay based on the hydrolysis of 4-nitrophenyl acetate, and the amount of lipase units adsorbed onto the papers was estimated by subtraction. In summary, the lipase solution after the removal of the paper was mixed with the buffer solution that was used for washing the paper. Then the mixture was diluted 11.4-fold with fresh buffer solution. Subsequently, the lipase solution (2.85 ml) was added to an acetonitrile solution of 4-nitrophenyl acetate (1.0 mM, 0.15 ml). The reaction progress of the hydrolysis of 4-nitrophenyl acetate to produce 4-nitrophenol was monitored at room temperature by UV-Vis absorption spectroscopy (V-670 spectrophotometer, JASCO); the enzymatic activity of the lipase solution was determined by measuring the change in absorbance at 400 nm, which was derived from 4-nitrophenol, as a function of time. The original lipase solution before soaking treatment of the paper samples was also subjected to the colorimetric assay, and the amount of lipase units adsorbed onto the papers was estimated by subtraction.

Nonaqueous transesterification reaction

The nonaqueous transesterification reaction was carried out in both batch and flow modes. In batch mode, (*R*,*S*)-1-phenylethanol (0.41 mmol) and vinyl acetate (0.62 mmol) were dissolved in isopropyl ether (10 ml). A piece of immobilized lipase on original or methacrylate-modified paper (33 mm in diameter) was then immersed in the solution. The reaction was carried out in a closed glass vial (SV-50A, NICHIDEN-RIKA GLASS Co. Ltd., 40 mm in diameter, 75 mm in height) at 23 °C with and without continuous stirring by an orbital platform shaker. At a given time, the reaction solution was analyzed to determine the concentration of the desired product, 1-phenylethylacetate, using a gas chromatograph with a (GC)-flame ionization detector, equipped with a TC-1 column (0.25 mm × 30 m, GL Sciences Inc.). As a control, free lipase powders were also subjected to the performance test. One unit of lipase activity (U) was defined as

the amount of enzyme required to produce 1 μmol of 1-phenylethylacetate per minute, which was determined on the basis of the amount produced over 1 h. To evaluate enantioselectivity, (*R*)-1-phenylethanol or (*S*)-1-phenylethanol was used as a substrate instead of (*R*,*S*)-1-phenylethanol. Reusability of immobilized lipase on methacrylate-modified paper was evaluated by a three-cycle test; the sequential procedures of the transesterification reaction (5 h), thorough washing with isopropyl ether and drying at room temperature were conducted in each cycle of the test. In flow mode, immobilized lipase on methacrylate-modified paper was cut into fourteen circular discs (9.0 mm in diameter) and the lipase content set equal to that used in the batch mode tests. The papers were vertically stacked (*ca.* 3.1 mm in thickness) and tightly packed into a syringe (Terumo syringe SS-02LZ, TERUMO, *ca.* 9.1 mm in diameter) equipped with a silicon tube. Subsequently, the substrate solution was fed into the paper layer at a constant flow rate of 0.1–2.0 ml min^{-1} using a syringe pump (Econoflo, Harvard Apparatus). Productivity was calculated as the amount of product (1-phenylethylacetate) per minute per mg lipase protein.

Analyses

Fourier transform infrared attenuated total reflection (FT-IR/ATR) spectra were obtained using a FT/IR-6100 instrument (JASCO). A diamond prism was used as an internal reflecting element for FT-IR/ATR analysis. X-ray fluorescence (XRF) analysis was carried out using a MESA-500 apparatus (HORIBA). Surface observation of as-prepared papers was conducted using a scanning electron microscope (SEM, S-4000, Hitachi, Ltd.) after osmium sputtering. X-ray diffraction (XRD) patterns were recorded using a Rigaku RINT 2000 with Ni-filtered Cu$K\alpha$ radiation ($\lambda = 1.5418$ Å) and scanning angle (2θ) in the range 10–30°, at 40 kV voltage and 40 mA current. For tensile strength measurements, paper samples were cut into rectangular pieces (5 mm in width, 20 mm in length). Tensile strengths and Young's moduli of the papers were evaluated at 23 °C and 50% relative humidity using a Shimadzu EZ-TEST instrument equipped with a 500 N load cell (pulling rate: 1.0 mm min^{-1}, span length: 10 mm). Wet strengths were measured after soaking in deionized water for 2.5 h. The surface hydrophobicity of the papers was evaluated by measuring the contact angle of water with a DropMaster 500 contact angle meter (Kyowa Interface Science Co. Ltd.) using the sessile drop technique.

Results and discussion

Preparation and characterization of methacrylate-modified paper

In situ modification of cellulose paper with methacryloxy groups was carried out using a silane coupling technique. 3-(Trimethoxysilyl)propyl methacrylate used as a silane coupling agent in this study was insoluble in pure water. Therefore, it was added to an acetic acid aqueous solution (pH = *ca.* 4), and the mixture was vigorously stirred for 30 min. Then, methoxy groups of 3-(trimethoxysilyl)propyl methacrylate were hydrolyzed to form silanol groups (Si–OH) and to afford the aqueous solution. Subsequently, a piece of cellulose paper was immersed in the resulting solution for 2 h, followed by evaporation of an acetic acid aqueous solution at 40 °C for 3 h under reduced pressure, thermal treatment and washing with ethanol. Fig. 1 profiles the FT-IR spectra of the original cellulose paper and the cellulose paper treated with 3-(trimethoxysilyl)propyl methacrylate. When the cellulose paper was treated with 3-(trimethoxysilyl)propyl methacrylate, the characteristic bands derived from C=C and C=O stretching appeared at *ca.* 1640 cm^{-1} and *ca.* 1720 cm^{-1}, respectively (Fig. 1b). In addition, CH_3 and CH_2 stretching vibration bands were observed at *ca.* 2950 cm^{-1} and *ca.* 2930 cm^{-1}, respectively.[48] These results indicate the presence of methacryloxy groups. As shown in Fig. 2, XRF spectra confirmed introduction of Si to the paper after treatment. Thus, it was suggested that cellulose paper was successfully modified with methacryloxy groups by the silane coupling technique. Previous reports concerning the silane coupling treatment of cellulose[37,49] suggest that the successful direct introduction of methacryloxy groups into cellulose paper was due to the condensation reaction between Si–OH of silane coupling agents and C–OH of cellulose. Fig. 3 shows SEM images of original and methacrylate-modified paper. The paper-specific interconnected porous microstructure remained almost unchanged even after the silane coupling treatment. However, the surface morphology of the cellulose fibers changed after the modification of methacryloxy groups, suggesting that silane coupling agents were coated on their surfaces. In addition, XRD analyses confirmed that the crystal structure of cellulose I (ref. 50) was maintained after silane coupling (see ESI, Fig. S2†). Thus, these results suggest that methacryloxy groups were introduced into the crystal surfaces of cellulose fibers.

Table 1 displays the physical properties of unmodified and methacrylate-modified cellulose paper. The weight of cellulose paper increased by *ca.* 27 mg after the silane coupling treatment, indicating that the content of silane coupling agents in the paper was about 24 wt%. Then the methacrylate content in the paper was roughly estimated at *ca.* 1.15 mmol g^{-1} by the change in paper weight. Methacrylate-modified paper was lightweight (*ca.* 0.6 g cm^{-3}). In addition, a *ca.* 20 μm increase in paper thickness was observed, possibly caused by coating of silane coupling agents on the cellulose fiber surfaces. The dry strength of cellulose paper is derived from intra- and inter-fiber hydrogen bonds

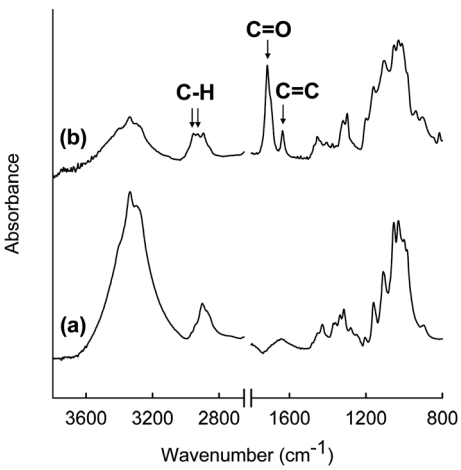


Fig. 1 FT-IR spectra of (a) original cellulose paper and (b) cellulose paper treated with 3-(trimethoxysilyl)propyl methacrylate.

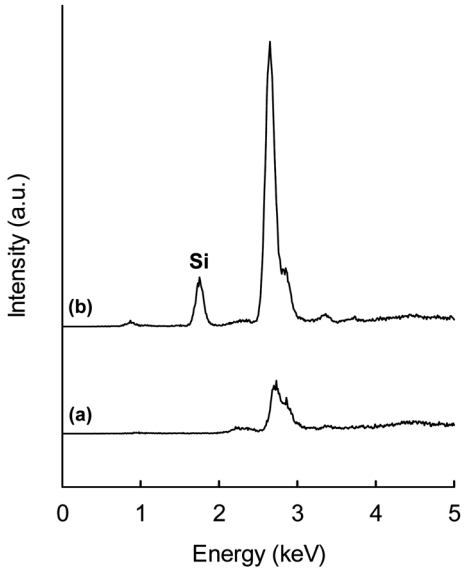


Fig. 2 XRF spectra of (a) original cellulose paper and (b) cellulose paper treated with 3-(trimethoxysilyl)propyl methacrylate.

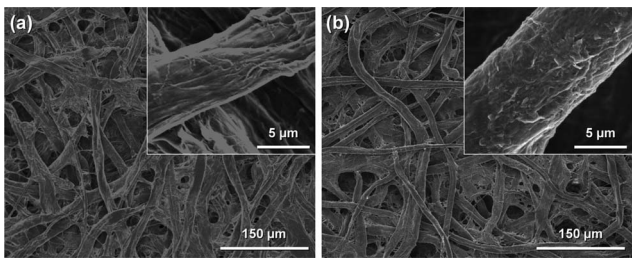


Fig. 3 SEM images of (a) original paper and (b) methacrylate-modified paper.

between the OH groups of cellulose. In the wet condition, however, water molecules intrude into cellulose paper and break hydrogen bonds, resulting in a fatal decrease in the paper strength. As shown in Table 1, methacrylate-modified paper had a higher physical strength, including Young's modulus and tensile strength, than the original paper, regardless of dry or wet state. It is notable that the wet tensile strength of methacrylate-modified paper was more than 7 times higher than that of original paper, leading to good practical utility even in water. This possibly resulted from the formation of bridges between cellulose fibers and silane coupling agents. In other words, silane coupling agents acted as cross-linkers by providing both condensation with the C–OH groups of cellulose and self-condensation between Si–OH groups, leading to the improvement of the physical strength of the paper. Fig. 4 shows optical images of water droplets on original and methacrylate-modified paper. The contact angle of water droplets on cellulose paper increased from 0° to *ca.* 110° with silane coupling, indicating a dramatic improvement in hydrophobicity. This result can be ascribed to hydrophobic moieties, the propyl and methacryloxy groups of silane coupling agents (see ESI, Fig. S1†), introduced into hydrophilic cellulose paper. Thus, the silane coupling treatment of cellulose paper is a facile and useful technique for introducing functional groups and improving physical strength and hydrophobicity.

Immobilization of lipase on methacrylate-modified paper

Immobilization of lipase on cellulose paper was carried out by a simple soaking treatment with an aqueous suspension of crude lipase powders. A piece of original or methacrylate-modified paper was immersed in a lipase solution with insoluble components contained in crude lipase powders. Since methacrylate-modified paper had some degree of hydrophobicity, it was forcibly immersed in the suspension. Subsequently, the mixture was stirred by using an orbital platform shaker. During the soaking treatment for 12 h, the paper samples were physically durable due to their acceptable wet strength. Insoluble components contained in crude lipase powders may clog the paper pores and disturb the immobilization of lipase. However, methacrylate-modified paper achieved effective immobilization of lipase even in the presence of the insoluble components; the immobilization yield of lipase on methacrylate-modified paper reached *ca.* 95%, while that on original paper was *ca.* 30%. The immobilized lipase on the paper was thoroughly washed with phosphate buffer solution at a stirring rate of 150 rpm for 3 h to remove unwanted insoluble components adsorbed onto the paper. The relatively hydrophobic nature of lipases[17,18] suggests that lipases were effectively attached to the hydrophobic surfaces of methacrylate-modified paper due to hydrophobic interaction. The modification of cellulose paper with methacryloxy groups therefore contributed to the effective immobilization of lipase.

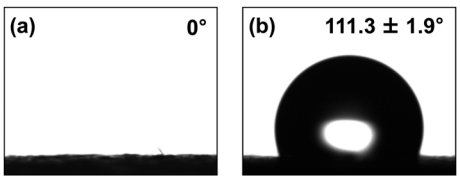


Fig. 4 Optical images and contact angles of water droplets on (a) original paper and (b) methacrylate-modified paper.

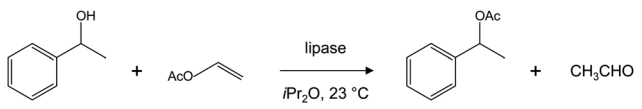
Table 1 Physical properties of unmodified and methacrylate-modified cellulose paper

			Young's modulus/GPa		Tensile strength/MPa	
	Weight/mg	Thickness/μm	Dry[a]	Wet	Dry[a]	Wet
Unmodified paper[b]	87.8 ± 0.8	201.3 ± 4.9	0.37 ± 0.06	0.04 ± 0.01	6.71 ± 0.29	0.76 ± 0.09
Methacrylate-modified paper	115.2 ± 2.8	223.3 ± 1.7	0.56 ± 0.04	0.17 ± 0.02	10.10 ± 0.42	5.79 ± 0.53

[a] Dry condition: 23 °C, 50% relative humidity. [b] Unmodified paper was prepared by the similar procedure as methacrylate-modified paper in the absence of any silane coupling agent, and the data were referred to ref. 37.

Nonaqueous transesterification by immobilized lipase on methacrylate-modified paper

In recent years, lipase-catalyzed transesterification reactions in nonaqueous media have attracted much attention for use in production of various useful chemicals through organic synthesis.[3,51,52] For this reason, the immobilized lipase on methacrylate-modified paper was used in the nonaqueous transesterification reaction between 1-phenylethanol and vinyl acetate to produce 1-phenylethylacetate (Scheme 1). Fig. 5 and Table 2 show enzymatic activities in batch-mode nonaqueous transesterification of free lipase and the immobilized lipase on original paper and methacrylate-modified paper. Free lipase without supporting materials had a low specific activity (0.63). Although isopropyl ether used as a reaction solvent in this study has been reported to be relatively harmless for lipase activity as compared with polar organic solvents such as methanol, ethylacetate and pyridine,[53,54] free lipase would be easily aggregated even in isopropyl ether, leading to a poor specific activity. In the case of the immobilized lipase on methacrylate-modified paper, however, specific activity was significantly improved at 25.3, which is *ca.* 40-fold higher than that of free lipase. Immobilized lipase on original paper had poor specific activity (0.31) and methacrylate-modified paper without lipase had no catalytic activity for this reaction. Many researchers have reported that hydrophobic solid supports allow effective interaction with the large hydrophobic pocket surrounding the catalytic site of lipases,[15,17–20] leading to hyperactivation of lipases *via* interfacial activation, *i.e.* the formation of a suitable open structure for efficient contact with substrates.[17,18] Thus, these results suggest that the hydrophobicity of the methacrylate-modified paper support (Fig. 4) contributes to the improvement of lipase activity for nonaqueous transesterification. In other words, lipase activity improved by the immobilization on methacrylate-modified paper due to hyperactivation of lipases and suppression of aggregate formation, leading to a large difference of activities between free and immobilized lipase. The specific activity of the immobilized lipase on methacrylate-modified paper (25.3) was comparable with those of the immobilized lipase on other supporting materials previously reported,[10] such as methacrylate-modified porous ceramics (37.2), diatomaceous earth (7.6), glass beads (0.8) and synthetic resins (0.4). Fig. 6 shows an enantioselectivity of the immobilized lipase on methacrylate-modified paper. When (*R*)-1-phenylethanol was used as a substrate, the specific activity reached 28.2. In the case of (*S*)-1-phenylethanol, however, the transesterification reaction did not progress at all. These results indicate an acceptable enantioselectivity of the immobilized lipase on methacrylate-modified paper for (*R*)-1-phenylethanol, in common with other immobilized lipases previously reported.[10,55,56] The advantages of paper as a support over conventional materials include its excellent practical utility, in that it is lightweight, flexible and easy to handle. In practice, the immobilized lipase on methacrylate-modified paper was easily recoverable after the performance test and was reusable without significant decrease in a specific activity (Table 2). Thus, the immobilization of lipase on methacrylate-modified paper led to the improvement of activity and reusability for nonaqueous transesterification, without notable loss of enantioselectivity.



Scheme 1 Lipase-catalyzed nonaqueous transesterification between 1-phenylethanol and vinyl acetate to produce 1-phenylethylacetate.

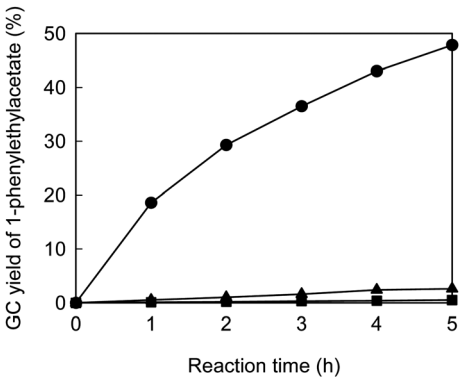


Fig. 5 Time course of GC yield of 1-phenylethylacetate in batch mode: free lipase (triangles) and immobilized lipase on original paper (squares) and methacrylate-modified paper (circles). Reaction temperature: 23 °C. Stirring rate: 150 rpm.

Flow reaction through an interconnected porous microstructure of immobilized lipase on methacrylate-modified paper

Flow-type catalytic reactions have received increasing attention for the continuous production of target substances. In accordance with this trend, there is growing interest in microstructured reactors[57–61] such as microchannels[22,23,29] and monolith foams,[11,62] which contain micrometre-scale open paths for fluids, because they allow fast mixing of reactants through small pores, leading to high reaction efficiency. In this study, the immobilized lipase on methacrylate-modified paper was tested in flow mode by taking advantage of the paper-specific interconnected porous microstructure (Fig. 3). Fig. 7 compares the productivities of 1-phenylethylacetate in batch and flow modes. The immobilized lipase on methacrylate-modified paper demonstrated acceptable permeability of the substrate solution within a flow rate of 2.0 ml min^{-1} possibly due to its highly porous structure, and was more effective in flow mode than in batch mode, with a maximum productivity recorded at a flow rate of 1.0 ml min^{-1} that was *ca.* 2-fold higher than that in batch mode at a stirring rate of 300 rpm. In the case of batch processes, which were conducted in a closed glass vial (40 mm in diameter, 75 mm in height), the millimetre-scale diffusion length of the reactants possibly results in a relatively low productivity because the accessibility of the reactants to the immobilized lipase on the paper would be insufficient, even at a stirring rate of 300 rpm. On the other hand, the flow reaction proceeded through the interconnected micrometre-scale pores of the paper. Such short diffusion paths inside the paper would provide fast mixing of the reactants and effective transport of the reactants to the catalytically active sites of lipases, leading to a high productivity. Cellulose is highly stable to most solvents and has both a hydrophilic and a lipophilic nature.[63–65] These unique properties are advantageous for use in

Table 2 Enzymatic activities in batch-mode nonaqueous transesterification of free lipase and lipase immobilized on original paper and on methacrylate-modified cellulose paper. Reaction temperature: 23 °C. Stirring rate: 150 rpm

Supporting material	Lipase protein content/μg	U[a]/μmol product per min	Specific activity/U per mg protein
None	56.0	0.035	0.63
Original paper	16.2	0.005	0.31
Methacrylate-modified paper	52.6	1.33	25.3(22.5[b])

[a] One unit of lipase activity (U) was defined as the amount of enzyme required to produce 1 μmol of 1-phenylethylacetate per minute. [b] Specific activity at the third cycle.

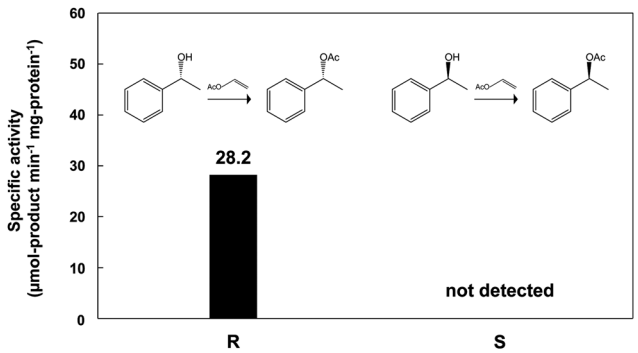


Fig. 6 Enantioselectivity of immobilized lipase on methacrylate-modified paper. Reaction temperature: 23 °C. Stirring rate: 150 rpm.

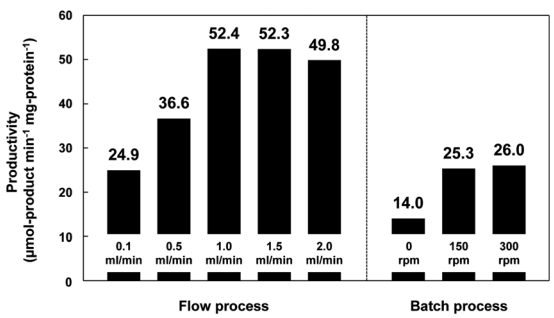


Fig. 7 Productivity of 1-phenylethylacetate in batch and flow processes using lipase immobilized on methacrylate-modified paper. Reaction temperature: 23 °C. Stirring rates in batch mode: 0, 150, and 300 rpm. Flow rates in flow mode: 0.1, 0.5, 1.0, 1.5, and 2.0 ml min^{-1}.

a variety of reaction systems. Thus, cellulose paper has potential applicability as a new class of microstructured reactor with excellent practical utility and paper-immobilized enzymes are expected to be promising catalytic materials for the continuous production of useful chemicals.

Conclusions

Cellulose paper was successfully modified with methacryloxy groups by a silane coupling technique and the as-prepared paper was used as a support material for the immobilization of lipase. The immobilized lipase on methacrylate-modified paper had a high degree of practical utility and demonstrated high catalytic activity, selectivity and reusability for the nonaqueous transesterification reaction. This indicates that the methacryloxy groups introduced into the cellulose paper were effective for the immobilization and hyperactivation of lipases. In addition, the paper-specific porous microstructure allowed higher productivity for continuous flow reaction systems than in batch reaction, possibly through provision of favorable diffusion paths for the reactants. The additional advantages of cellulose paper over other support materials may include sustainability and high mass productivity, while the silane coupling technique for the introduction of functional groups into cellulose paper is facile and versatile. Thus, chemically modified cellulose paper is a green support material that provides both excellent practical utility and favorable reaction fields for enzymes and other catalysts.

Acknowledgements

This research was supported by a Research Fellowship for Young Scientists from the Japan Society for the Promotion of Science (H. K.).

References

1 K. M. Koeller, *Nature*, 2001, **409**, 232–240.
2 A. Schmid, J. S. Dordick, B. Hauer, A. Kiener, M. Wubbolts and B. Witholt, *Nature*, 2001, **409**, 258–268.
3 A. Zaks and A. M. Klibanov, *Proc. Natl. Acad. Sci. U. S. A.*, 1985, **82**, 3192–3196.
4 M. L. Foresti and M. L. Ferreira, *Catal. Today*, 2005, **107/108**, 23–30.
5 G. D. Yadav and P. S. Lathi, *J. Mol. Catal. B: Enzym.*, 2004, **27**, 113–119.
6 F. Yagiz, D. Kazan and A. N. Akin, *Chem. Eng. J.*, 2007, **134**, 262–267.
7 A. M. Klibanov, *Trends Biotechnol.*, 1997, **15**, 97–101.
8 R. M. Blanco, P. Terreros, M. Fernández-Pérez, C. Otero and G. Diaz-González, *J. Mol. Catal. B: Enzym.*, 2004, **30**, 83–93.
9 Y.-X. Bai, Y.-F. Li, Y. Yang and L.-X. Yi, *J. Biotechnol.*, 2006, **125**, 574–582.
10 M. Kamori, T. Hori, Y. Yamashita, Y. Hirose and Y. Naoshima, *J. Mol. Catal. B: Enzym.*, 2000, **9**, 269–274.
11 L. Huang and Z.-M. Cheng, *Chem. Eng. J.*, 2008, **144**, 103–109.
12 S. Shah, K. Solanki and M. N. Gupta, *Chem. Cent. J.*, 2007, **1**, 30–35.
13 S. Sakai, Y. Liu, T. Yamaguchi, R. Watanabe, M. Kawabe and K. Kawakami, *Biotechnol. Lett.*, 2010, **32**, 1059–1062.
14 N. N. Gandhi, V. Vijayalakshmi, S. B. Sawant and J. B. Joshi, *Chem. Eng. J. Biochem. Eng. J.*, 1996, **61**, 149–156.
15 C. Mateo, J. M. Palomo, G. Fernandez-Lorente, J. M. Guisan and R. Fernandez-Lafuente, *Enzyme Microb. Technol.*, 2007, **40**, 1451–1463.
16 W. Tischer and F. Wedekind, *Top. Curr. Chem.*, 1999, **200**, 95–126.
17 A. Bastida, P. Sabuquillo, P. Armisen, R. Fernandez-Lafuente, J. Huguet and J. M. Guisan, *Biotechnol. Bioeng.*, 1998, **58**, 486–493.
18 R. Fernandez-Lafuente, P. Armisen, P. Sabuquillo, G. Fernandez-Lorente and J. M. Guisan, *Chem. Phys. Lipids*, 1998, **93**, 185–197.
19 M. Petkar, A. Lali, P. Caimi and M. Daminati, *J. Mol. Catal. B: Enzym.*, 2006, **39**, 83–90.
20 G. Fernandez-Lorente, Z. Cabrera, C. Godoy, R. Fernandez-Lafuente, J. M. Palomo and J. M. Guisan, *Process Biochem.*, 2008, **43**, 1061–1067.

21 G. A. Kovalenko, O. V. Komova, A. V. Simakov, V. V. Khomov and N. A. Rudina, *J. Mol. Catal. A: Chem.*, 2002, **182–183**, 73–80.
22 J. Kaneno, R. Kohama, M. Miyazaki, M. Uehara, K. Kanno, M. Fujii, H. Shimizu and H. Maeda, *New J. Chem.*, 2003, **27**, 1765–1768.
23 M. Miyazaki, J. Kanenoc, R. Kohama, M. Uehara, K. Kanno, M. Fujii, H. Shimizu and H. Maeda, *Chem. Eng. J.*, 2004, **101**, 277–284.
24 K. M. de Lathouder, J. Bakker, M. T. Kreutzer, F. Kapteijn, J. A. Moulijn and S. A. Wallin, *Chem. Eng. Sci.*, 2004, **59**, 5027–5033.
25 T. Debeche, C. Marmet, L. Kiwi-Minsker, A. Renken and M.-A. Juillerat, *Enzyme Microb. Technol.*, 2005, **36**, 911–916.
26 K. M. de Lathouder, T. Marques Fló, F. Kapteijn and J. A. Moulijn, *Catal. Today*, 2005, **105**, 443–447.
27 S. Kataoka, A. Endo, M. Oyama and T. Ohmori, *Appl. Catal., A*, 2009, **359**, 108–112.
28 F. Costantini, E. M. Benetti, D. N. Reinhoudt, J. Huskens, G. J. Vancso and W. Verboom, *Lab Chip*, 2010, **10**, 3407–3412.
29 Y. Asanomi, H. Yamaguchi, M. Miyazaki and H. Maeda, *Molecules*, 2011, **16**, 6041–6059.
30 D. Klemm, B. Heublein, H.-P. Fink and A. Bohn, *Angew. Chem., Int. Ed.*, 2005, **44**, 3358–3393.
31 M. B. Turner, S. K. Spear, J. D. Holbrey, D. T. Daly and R. D. Rogers, *Biomacromolecules*, 2005, **6**, 2497–2502.
32 M. Nogi, S. Iwamoto, A. N. Nakagaito and H. Yano, *Adv. Mater.*, 2009, **21**, 1595–1598.
33 H. Fukuzumi, T. Saito, T. Iwata, Y. Kumamoto and A. Isogai, *Biomacromolecules*, 2009, **10**, 162–165.
34 S. Egusa, S. Yokota, K. Tanaka, K. Esaki, Y. Okutani, Y. Ogawa, T. Kitaoka, M. Goto and H. Wariishi, *J. Mater. Chem.*, 2009, **19**, 1836–1842.
35 L. Ye, C. D. M. Filipe, M. Kavoosi, C. A. Haynes, R. Pelton and M. A. Brook, *J. Mater. Chem.*, 2009, **19**, 2189–2198.
36 H. Koga, E. Tokunaga, M. Hidaka, Y. Umemura, T. Saito, A. Isogai and T. Kitaoka, *Chem. Commun.*, 2010, **46**, 8567–8569.
37 H. Koga, T. Kitaoka and A. Isogai, *J. Mater. Chem.*, 2011, **21**, 9356–9361.
38 R. J. Moon, A. Martini, J. Nairn, J. Simonsen and J. Youngblood, *Chem. Soc. Rev.*, 2011, **40**, 3941–3994.
39 T. Saito, T. Uematsu, S. Kimura, T. Enomae and A. Isogai, *Soft Matter*, 2011, **7**, 8804–8809.
40 A. Azetsu, H. Koga, A. Isogai and T. Kitaoka, *Catalysts*, 2011, **1**, 83–96.
41 H. Koga, A. Azetsu, E. Tokunaga, T. Saito, A. Isogai and T. Kitaoka, *J. Mater. Chem.*, 2012, **22**, 5538–5542.
42 H. Koga, T. Kitaoka and H. Wariishi, *Chem. Commun.*, 2008, 5616–5618.
43 H. Koga, T. Kitaoka and H. Wariishi, *J. Mater. Chem.*, 2009, **19**, 5244–5249.
44 H. Koga, T. Kitaoka, M. Nakamura and H. Wariishi, *J. Mater. Sci.*, 2009, **44**, 5836–5841.
45 H. Koga, Y. Umemura, A. Tomoda, R. Suzuki and T. Kitaoka, *ChemSusChem*, 2010, **3**, 604–608.
46 A. Salis, M. Pinna, M. Monduzzi and V. Solinas, *J. Mol. Catal. B: Enzym.*, 2008, **54**, 19–26.
47 P. J. O'Connell and J. Varley, *Biotechnol. Bioeng.*, 2001, **74**, 264–269.
48 G. Socrates, *Infrared and Raman Characteristic Group Frequencies: Tables and Charts*, John Wiley & Sons, Ltd., Chichester, 2001.
49 M. Abdelmouleh, S. Boufi, M. N. Belgacem, A. P. Duarte, A. Ben Salah and A. Gandini, *Int. J. Adhes. Adhes.*, 2004, **24**, 43–54.
50 R. H. Atalla and S. C. Nagel, *Science*, 1974, **185**, 522–523.
51 S. Shah, S. Sharma and M. N. Gupta, *Energy Fuels*, 2004, **18**, 154–159.
52 T. Itoh, Y. Matsushita, Y. Abe, S.-H. Han, S. Wada, S. Hayase, M. Kawatsura, S. Takai, M. Morimoto and Y. Hirose, *Chem.–Eur. J.*, 2006, **12**, 9228–9237.
53 C. Laane, S. Boeren, K. Vos and C. Veeger, *Biotechnol. Bioeng.*, 1987, **30**, 81–87.
54 Y. Hu and L.-K. Ju, *Biotechnol. Prog.*, 2003, **19**, 303–311.
55 P. C. Jesus, M. C. Rezende and M. G. Nascimento, *Tetrahedron: Asymmetry*, 1995, **6**, 63–66.
56 P. Lozano, E. García-Verdugo, N. Karbass, K. Montague, T. de Diego, M. I. Burguete and S. V. Luis, *Green Chem.*, 2010, **12**, 1803–1810.
57 L. Kiwi-Minsker and A. Renken, *Catal. Today*, 2005, **110**, 2–14.
58 A. Cybulski and J. A. Moulijn, *Structured Catalysts and Reactors*, Taylor & Francis, Boca Raton, 2006.
59 B. P. Mason, K. E. Price, J. L. Steinbacher, A. R. Bogdan and D. T. McQuade, *Chem. Rev.*, 2007, **107**, 2300–2318.
60 J. Yoshida, A. Nagaki and T. Yamada, *Chem.–Eur. J.*, 2008, **14**, 7450–7459.
61 J. M. Bolivar, J. Wiesbauer and B. Nidetzky, *Trends Biotechnol.*, 2009, **29**, 333–342.
62 A. El Kadib, R. Chimenton, A. Sachse, F. Fajula, A. Galarneau and B. Coq, *Angew. Chem., Int. Ed.*, 2009, **48**, 4969–4972.
63 M. Jarvis, *Nature*, 2003, **426**, 611–612.
64 Y. Nishiyama, P. Langan and H. Chanzy, *J. Am. Chem. Soc.*, 2002, **124**, 9074–9082.
65 Y. Nishiyama, J. Sugiyama, H. Chanzy and P. Langan, *J. Am. Chem. Soc.*, 2003, **125**, 14300–14306.