Lipolytic Enzymes with Improved Activity and Selectivity upon Adsorption on Polymeric Nanoparticles

Cleofe Palocci,*,† Laura Chronopoulou,† Iole Venditti,† Enrico Cernia,† Marco Diociaiuti,‡ Ilaria Fratoddi,† and Maria Vittoria Russo†

Department of Chemistry, University of Rome "La Sapienza", P.le A.Moro 5, 00185 Rome, Italy, and Dipartimento di Tecnologie e Salute, Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Rome, Italy

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Nanostructured polystyrene (PS) and polymethylmethacrylate (PMMA) were used as carriers for the preparation of bioconjugates with lipolytic enzymes, such as *Candida rugosa* lipase (CRL) and *Pseudomonas cepacia* lipase (PCL). Simple addition of the lipase solution to the polymeric nanoparticles under protein-friendly conditions (pH 7.6) led to the formation of polymer−enzyme bioconjugates. Energy filtered-transmission electron microscopy (EF-TEM) performed on immuno-gold labeled samples revealed that the enzyme preferentially binds to the polymer nanoparticles and that the binding does not affect the nanostructured features of the carriers. The studies performed on the activity of the bioconjugates pointed out that the lipases adsorbed onto polymeric nanoparticles show an improved performance in terms of activity and selectivity with respect to those shown by lipases adsorbed on the same non-nanostructured carriers. The residual activities of CRL and PCL immobilized on nanostructured PMMA and PS reached 60% and 74%, respectively. Moreover, we found that enantioselectivity and pH and thermal stability increase upon immobilization. These results highlight the fact that new protein conformers with improved enantioselectivity stabilized after adsorption on nanoparticles are obtained. On the basis of the chemical structures of the selected polymers and the slopes of the adsorption isotherms, a hydrophobic binding model for lipase/nanostructured polymers is suggested.

Introduction

Lipases are frequently employed enzymes as they are commonly used for the synthesis of enantioenriched compounds for industrial applications, for clinical analysis, and for polymerization reactions.[1−4] With the aim of improving their stability, separation and reuse, and continuous operation, their immobilization has been studied extensively. Often the low catalytic efficiency of these biocatalysts can limit their use in the development of large scale bioprocessing and cannot compete with traditional chemical processing.[5−8] A novel approach to improving the efficiency of immobilized lipases is to manipulate the structure of carrier materials.

Nonporous carrier materials, to whose surfaces the enzymes are attached, are subject to minimum diffusional limitations.[9−12] However, enzyme loading per unit mass of support is usually considerably low. Alternatively, high enzyme loading can be achieved with porous materials.[7, 8,11−17] Porous materials, however, suffer much greater diffusional limitations.

To date, extensive efforts have been conducted to optimize the carrier's structure and to make more efficient biocatalysts.[14−16,18−22] In this regard, nanostructured materials will provide the upper limits in terms of balancing the contradictory issues, including surface area, mass transfer resistance, and effective enzyme loading.[17−23] Recently reported work in this area has revealed the great potential for the use of nonporous,[18−24] nanofibrous,[19−25] and nanoparticle[20,26−29] materials as a new class of carriers for biocatalysts. The effective enzyme loading on nanomaterials can be considerably high (e.g., it can reach over 10 wt % with particles smaller than 100 nm), and a large surface area per unit mass is also provided to facilitate reaction kinetics. Moreover, the nanoparticle conjugation with biomolecules is expected to modify the properties of the nanoparticles and their interactions with the environment (for example their solubility). There are several reports on the formation of hybrids made from gold nanoclusters (core) with polymers or biomolecules (shell), which are able to conjugate with enzymes,[30] and *vice versa*[31]. The presence of the metal, besides producing a biocompatible material, enhances the stability of the immobilized enzymes and allows an easy synthesis of the bioconjugate since it avoids the need to modify the polymer surface that is involved in the linkage of the enzyme−protein.

In this article, we demonstrate for the first time the direct assembly of *Candida rugosa* and *Pseudomonas cepacia* lipase molecules on polymethylmethacrylate (PMMA) and polystyrene (PS) nanoparticles obtained by emulsion polymerization techniques. Binding of the enzymatic proteins to the polymer nanoparticles occurs through noncovalent interactions and thus obviates the need for surface modification of the polymer spheres. In addition to the simplicity of the protocol for their preparation, the lipase−nanoparticle bioconjugates exhibit high retention of biocatalytic activity and significantly enhanced thermal and pH stability.

Experimental Procedures

Materials. Lipase (EC 3.1.1.3) from *Candida rugosa* (CRL), type VII 890 U/mg, (±)-1-phenylethanol, *R*-(+)-phenylethanol, *S*-(−)-phenylethanol, phenylacetate, vinylacetate, tributyrin, organic solvents analytical grade, bovine serum albumin (BSA, molecular mass: 67.000 Da),

* Corresponding author. Phone: +39-6-49913317. Fax: +39-6-490324. E-mail: cleofe.palocci@uniroma1.it.
† University of Rome "La Sapienza".
‡ Istituto Superiore di Sanità.

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2-morpholino-ethane sulfonic acid (MES), used as received, were purchased from Sigma-Aldrich. The other chemicals, the MMA (methylmethacrylate) monomer (Aldrich 99% pure), the S (styrene) monomer (Aldrich 99% pure), and potassium persulfate initiator (Aldrich 99.99% pure), were used as received. Lipase from *Pseudomonas cepacia* lipase (PCL) was kindly provided by Amano Pharmaceuticals Co. Ltd. (Nagoya, Japan). Monoclonal antibodies (MoAbs) BF11 against CRL were produced following the procedures described in a previous patent by some of the authors of the present article (PCT/EPO2/12149;Antimicrobial lipase antibodies, their nucleotidic and aminoacidic sequences and use thereof; n°3732 PTWO-A G/rb),[32] and anti-mouse IgG 5-nm gold coniugate was purchased from Sigma Chemical Co..

Preparation of the Carriers. PMMA and polystyrene nanospheres with diameters of 100, 200, and 500 nm, free of emulsifiers, were prepared according to emulsion polymerization methods described in the literature.[33] PS nanoparticles were separated from the reaction medium by repeating cycles of centrifugation/redispersion until no styrene monomers were spectrophotometrically detected in the supernatant. PMMA nanoparticles were obtained from the opalescent emulsion by filtration through a standard paper filter and then centrifuged and redispersed with deionized water five times to remove the unreacted monomer and toluene.

Protein Assay. The amount of lipase in solution was measured by the Bradford method.[34]

Adsorption Experiments. Adsorption experiments of CRL and PCL onto PMMA and PS nanoparticles were performed in pyrex tubes containing a known amount of polymer dispersed in 2 mL of a buffer solution (0.1 M phosphate buffer at pH 7.6) of lipase (50 mg/mL), under gentle shaking at controlled temperature (25 °C). The time course of lipase adsorption was followed for 3 h, and the samples were filtered through a cellulose nitrate filter membrane (Whatman, pore diameter 0.01−0.2 μm) to completely separate the particles.

The amount of immobilized enzyme was obtained by standard Bradford assay of the original lipase solutions, the supernatants, and the washing solutions after immobilization. The protein concentration was determined spectrophotometrically with the adsorption peak at 595 nm.

Desorption Studies. The immobilization of CRL and PCL on nanostructured PMMA and PS nanoparticles was tested by surface desorption experiments. The lipase-covered nanoparticles were thus incubated at different temperatures (20 °C and 40 °C) and suspended in fixed amounts of phosphate buffer solution (0.1 M, pH 7.6) equilibrated to ensure a steady state. The supernatant was examined for desorbed lipases with the Bradford assay.

Lipolytic Assay of Free and Immobilized Lipases. The activity of free and immobilized lipase preparations was determined according to the standard hydrolysis assay. In standard conditions, the reaction mixture composed of 2.5 mL of phosphate buffer solution (PBS) at pH 7.6, 0.5 mL of tributyrin, and 0.1 mL of enzyme solution (50 mg/mL) was incubated at 37 °C under magnetic stirring (600 rpm) for 30 min. Hydrolysis was stopped by adding 3.0 mL of acetone/ethanol mixture 1:1, and the reaction mixture was titrated with 0.1 M NaOH in the presence of phenolphthalein as an indicator using an automatic titrator (Metrohm).

Esterase Activity Measurements. Esterase activity of CRL and PCL free and immobilized on nanostructured polymers were derived from the rate of the transesterification reaction of 1-phenylethanol (0.25 mM) with vinylacetate (1.25 mM) in organic solvents using 5 mg of immobilized lipase. The mixture was incubated at different temperatures for 6 h under magnetic stirring. Samples were taken for analysis at regular intervals.

Analytical Conditions. The progress of the reaction for ester production was followed by GC using a capillary column of polyphenylmethylsiloxane (MEGA 25 m × 0.53 mm ID) and a flame ionization detector, nitrogen as the carrier gas, at a pressure of 0.8 bar and 473 K for the detector. The oven temperature gradient was set in the range from 70 to 130 °C with a rate of 40 °C/min. The enantiomeric resolution was obtained employing two serial capillary columns: the first one achiral (OV-1: 25 m × 0.25 mm ID), the second one chiral (2,3-*O*-dimethyl-6-pentyl-β-cyclodextrin, 30% OV 1701, 25 m × 0.25 mm ID).

Lipase Adsorption Kinetics. The adsorption of CRL and PCL onto nanostructured PMMA and PS was performed with polymer dispersions of 50 mg/mL. Adsorption kinetics were carried out in 10 mL buffer solutions at constant pH and at controlled temperature (25 °C) by using a fixed lipase concentration. The time courses of CRL and PCL adsorptions on nanostructured polymers were followed for 4 h. The polymer/enzyme dispersions were centrifuged, and the amount of lipase in the supernatant was quantified by the Bradford method as described above. The amount of adsorbed lipase was then calculated by the difference between the amount of lipase in the solution before adsorption and the lipase remaining in the supernatant after the adsorption.

Evaluation of the Adsorption Isotherms. Adsorption isotherms were established at 25 °C in 10 mM phosphate buffer at pH 7.6. Known amounts of protein and carrier were mixed and incubated for 3 h. Thereafter, the mixture was filtered, and the protein concentration in the clear supernatant was measured by using the Bradford assay. The amount of adsorbed protein was determined from the difference between the protein concentration in solution before and after adsorption.

pH and Thermal Stability Measurements. The thermal stabilities of bound and free lipases were investigated by measuring their residual activities at 37 °C after incubating in buffer for 30 min at the desired temperature in the range of 30−70 °C at a fixed pH value. The pH stability of the free and immobilized lipases was assayed by immersing them in PBS in the pH range of 6−8 for 1 h at different temperatures and then assaying their residual activities.

Scanning Electron Microscopy (SEM) Analysis. Samples for SEM measurements were prepared by casting a drop of nanoparticle−lipase bioconjugate solution on silica support. Dried nanoparticles were analyzed at 15−20 kV by a scanning electron microscope (SEM-LEO 1450 VP) after metallization by gold coating.

Transmission Electron Microscopy (TEM)Analysis. Samples containing (i) PMMA nanoparticles with CRL and (ii) plain CRL as control were deposited onto thin amorphous carbon films (about 20 nm) supported by Cu grid (400 mesh) for transmission electron microscopy and air-dried.[35] Samples were preincubated for 5 min with 1% w/v bovine serum albumin (BSA) (Sigma Chemical Co., USA) in phosphate buffer (0.1 M, pH 7.0) and then incubated for 30 min at room temperature with the mouse monoclonal anti-CRL (Mo AB BF11) at a concentration of 40 μg/mL. After washing for 10 min by floating the grids on phosphate buffer drops containing 1% w/v BSA, samples were labeled with anti-mouse IgG 5-nm gold conjugate diluted 1:10 (Sigma Chemical Co., USA) for 30 min at room temperature. Finally, samples were washed with phosphate buffer and distilled water and air-dried. Samples were studied in a Zeiss 902 transmission electron microscope, operating at 80 kV, equipped with an electron energy loss filter. In order to enhance the contrast, the microscope was used in the electron spectroscopy imaging (ESI) mode filtering at $\Delta E = 0$ eV. The image acquisition was performed by a digital CCD camera model HSC2, 1k for 1k pixels, (PROSCAN GMBH, Germany), thermostatted by a Peltier cooler model WKL 230 (LAUDA GMBH, Germany). Image analysis and quantification was performed by a digital image analyzer analySIS 3.0 (SIS GMBH, Germany). This software allows one to enhance contrast and sharpness of the acquired images and to perform morphological quantification and statistics. The dimensional measurements were performed after a careful magnification calibration of the whole imaging system based on reference standards (cross grating and catalase crystal).

Results and Discussion

To examine the effect of particle size on lipase adsorption and kinetics, polystyrene and polymethylmethacrylate nanopar-

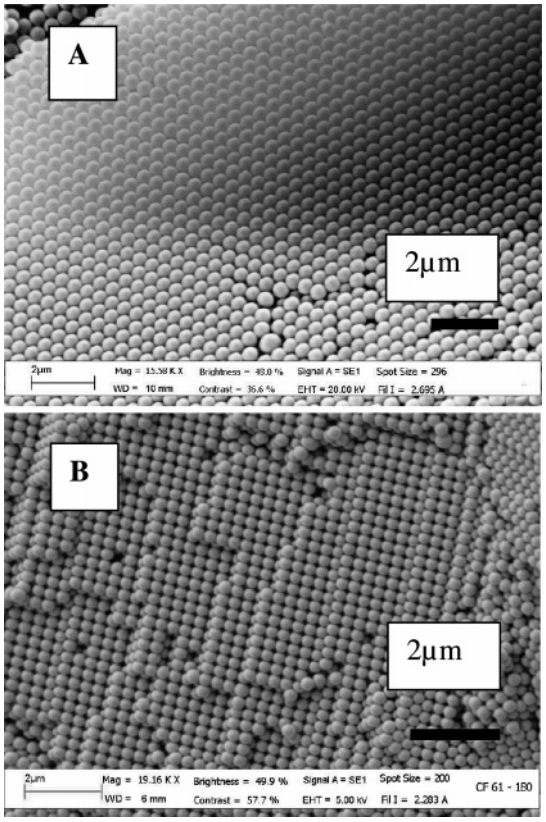

Figure 1. Scanning electron microscopy (SEM) images of the nanostructured PS (A) and PMMA (B), with an average diameter of 300 nm.

ticles were prepared on purpose by using a modified emulsion polymerization technique. Dimension control, very low polydispersion, and regular spherical shape were obtained by controlling the amount of emulsifier, the sorbent/water phase ratio, and reaction time.[33] Monodispersed nanoparticles of four different sizes were synthesized and selected as carriers for the current study.

SEM images of the particles were collected, showing that samples with average diameter ranging from 100 to 400 nm for PS and PMMA were obtained. Figure 1 shows the SEM images as an example of the two nanostructured polymers with 300 nm bead average diameter.

The energy filtered-transmission electron microscopy (EF-TEM) images shown in Figure 2 were obtained without any staining techniques. They easily allowed us to localize the CRL molecules, labeled with a gold particle of about 5 nm that appeared as a dark spot. In the absence of PMMA (panel B), the enzyme appeared uniformly distributed onto the flat amorphous carbon substrate, with a high concentration. Conversely, in the presence of PMMA nanoparticles (panel A), the background was nearly CRL free, and the majority of CRL decorated the PMMA nanoparticles. This observation clearly demonstrates that CRL binds to the PMMA nanoparticle.

As is well known, protein adsorption onto solid surfaces is controlled by the properties of the support surface, the nature of the protein molecule, and the solution conditions. The surfaces of CRL and PCL are complex in nature, with differences in characteristics such as the hydrophobicity of the different CRL isoenzymes. The absence of titrable groups on the surfaces of PS and PMMA makes the adsorption mechanism of CRL controlled by hydrophobic and van der Waals bond interactions, whereas the contribution of electrostatic interactions is likely to be very small. CRL and PCL were adsorbed onto PS and

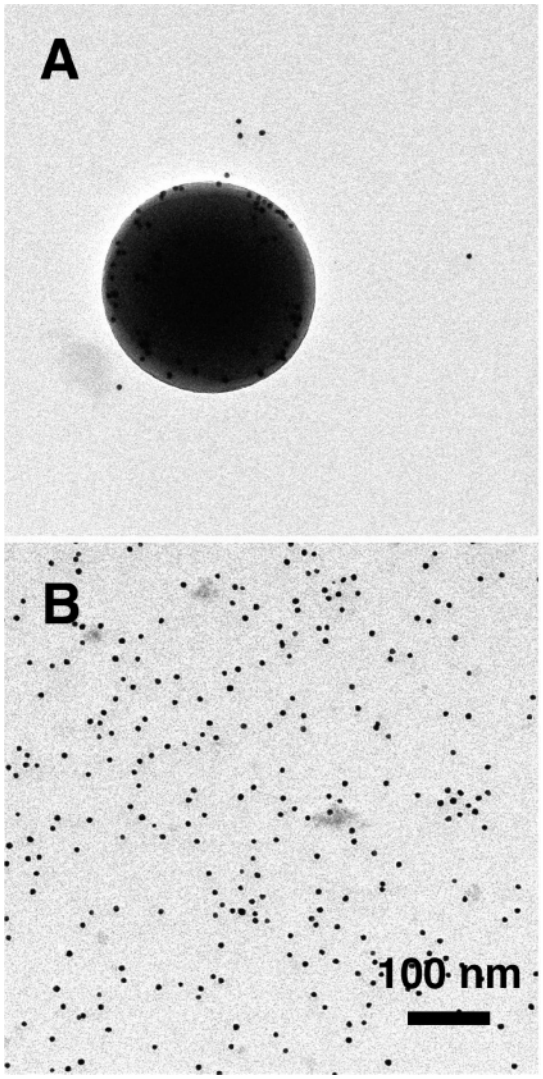

Figure 2. Energy filtered-transmission electron microscopy (EF-TEM) images of 5-nm immunolabeled CRL in the presence (panel A) and absence (panel B) of PMMA nanoparticles of about 200 nm.

PMMA nanoparticles via physical adsorption by using a fixed amount of solid nanoparticles and a solution (in 0.1 M PBS at pH 7.6) of the enzyme. Control experiments were carried out to confirm that the enzymes were firmly attached to the particles and that no free enzyme leached off the particles during the reaction. These experiments were based on the routine evaluation of enzymatic activity.

The enzyme loading does not seem to be influenced by the dimensions of the nanoparticles in the range of 150−400 nm; therefore, we chose to carry out all experiments with the particles of 300 nm average diameter. The time course of the adsorption of CRL and PCL onto PMMA and PS nanoparticles were evaluated. More than 80% of lipase molecules were adsorbed after 25 min of reaction time. Figure 3 reports the results that refer to the bioconjugate CRL-PMMA and is representative of all of the investigated bioconjugates. Adsorption of lipases onto the selected polymers is a fast process because of the high affinity of lipase molecules toward hydrophobic surfaces, as demonstrated by the authors in a previous work.[36] This experimental evidence confirms the hypothesis that the adsorption phenomena at the nanoparticle−enzyme interface is driven by hydrophobic forces. The most important aspect in lipase−nanoparticle bioconjugate systems is the retention of the biocatalytic activity after adsorption onto the surfaces of the

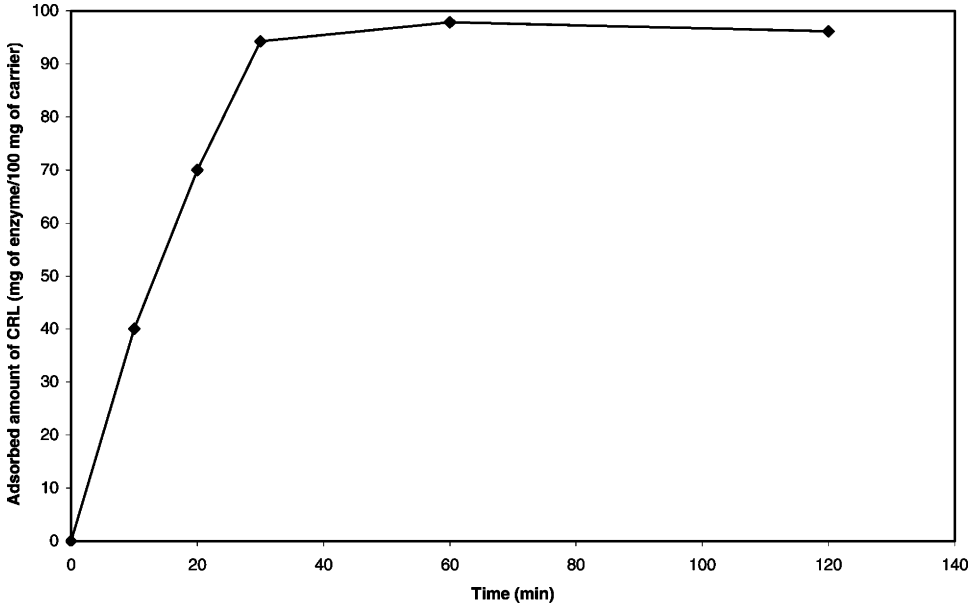

Figure 3. Adsorption kinetics of CRL on nanostructured PMMA at $T = 25$ °C. Reaction conditions: enzyme, 50 mg/mL; reaction volume, 2 mL; polymer = 100 mg; phosphate buffer, 0.1 M at pH 7.6; average diameter of nanoparticles, 300 nm.

Table 1. Activity of Free and Immobilized Lipases[a] under Standard Reaction Conditions

samples	specific activity (U/mg)	activity retention (%)
Free CRL	3.85	100
CRL on amorphous PMMA	0.77	20
CRL on nanostructured PMMA	2.31	60
CRL on amorphous PS	1.12	29
CRL on nanostructured PS	2.83	74
Free PCL	7.66	100
PCL on amorphous PMMA	2.46	32
PCL on nanostructured PMMA	5.13	67
PCL on amorphous PS	2.24	29
PCL on nanostructured PS	5.62	73

[a] Reaction conditions: 5 mg of enzyme; reaction volume, 2.5 mL; phosphate buffer, 0.1 M and 100 μL of tributyrin at pH 7.6; $T = 37$ °C; reaction time, 30 min; average diameter of nanoparticles, 300 nm.

nanoparticles. In our study, the specific activity was calculated using the amount of enzyme estimated from the calibration curve and compared with that of the free enzyme in solution under identical conditions. The specific activity of free and immobilized lipases are reported in Table 1. It is noteworthy that in comparison to the behavior of the immobilized enzymes on amorphous supports, CRL and PCL exhibit a significant increase in their activity when adsorbed on nanostructured polymers. CRL and PCL show retention activity values from 60% up to 74% when adsorbed on nanostructured PMMA and PS, respectively, whereas lipase adsorption on amorphous polymers gives retention activity values up to about 20−30%. These results can be explained by the remarkably high specific area of the nanostructured polymers, which can provide more potential reaction sites for lipase adsorption. Moreover, it was demonstrated that the activity of bioconjugates was not affected by pH and temperature variations. Figure 4a and b shows as an example the thermal and pH stabilities of free and immobilized CRL preparations. It can be seen that the free lipase lost its initial activity rapidly after the temperature reached $T = 50$ °C, while the immobilized lipase retains its initial activity (about 100%) after incubation for 30 min in the temperature range 30−60 °C. These results indicate that the thermal stability of immobilized lipases on nanostructured polymers is much higher than that of the free ones. The formation of physical interactions between the lipase and the polymeric carrier prevents conformational transitions of the enzyme at high temperature. As far as pH stabilities are concerned, there was no loss of activity of the immobilized lipases in the pH range from 6 to 8, while the free enzymes rapidly lost their activity up to 80% when the solutions reached pH ≥ 8.5, although their activity is higher than that of the bioconjugates.

Adsorption isotherms, in which the amount of proteins adsorbed per unit weight of sorbent is plotted against the protein concentration in solution, display different characteristics of the protein−sorbent interaction such as the adsorption affinity of the enzymes for the nanostructured carriers. Adsorption isotherms at 25 °C and at constant pH values were obtained after 1 h of incubation of CRL and PCL with the nanostructured PMMA and PS. Figure 5 shows as an example CRL adsorption mass and activity isotherms with a well-defined plateau of adsorption onto nanostructured PMMA for equilibrium lipase concentrations in solution of $C_{eq} = 2$ mg/mL. A Langmuir-type model (eq 1)[37] fitted both the mass and the activity trends of adsorbed CRL and PCL onto PMMA and PS nanoparticles.

$$Q_e = (K_L C_e)/(1 + a_L C_e) \quad (1)$$

The characterization of CRL and PCL in the adsorbed state (e.g., enzymatic activity test) requires one to know whether the enzymes are also immobilized on the particles at the different temperature employed for the activity tests. Resuspension of the bioconjugates in the phosphate buffer (0.2 M, pH 7.6) did not result in changes of the surface concentration of CRL and PCL. In the case of CRL adsorbed on nanostructured PMMA and PS, no lipase was detected in the supernatant as a function of reaction time and at different temperatures (20−40 °C). The strong hydrophobic character of the carriers and the presence of large hydrophobic domains on the surface of the CRL molecules probably hinders the desorption. The time course of PCL desorption from nanostructured polymers is also very slow, and the bioconjugate retains up to 80% of the initial enzyme activity (data not shown). These experimental results are in good

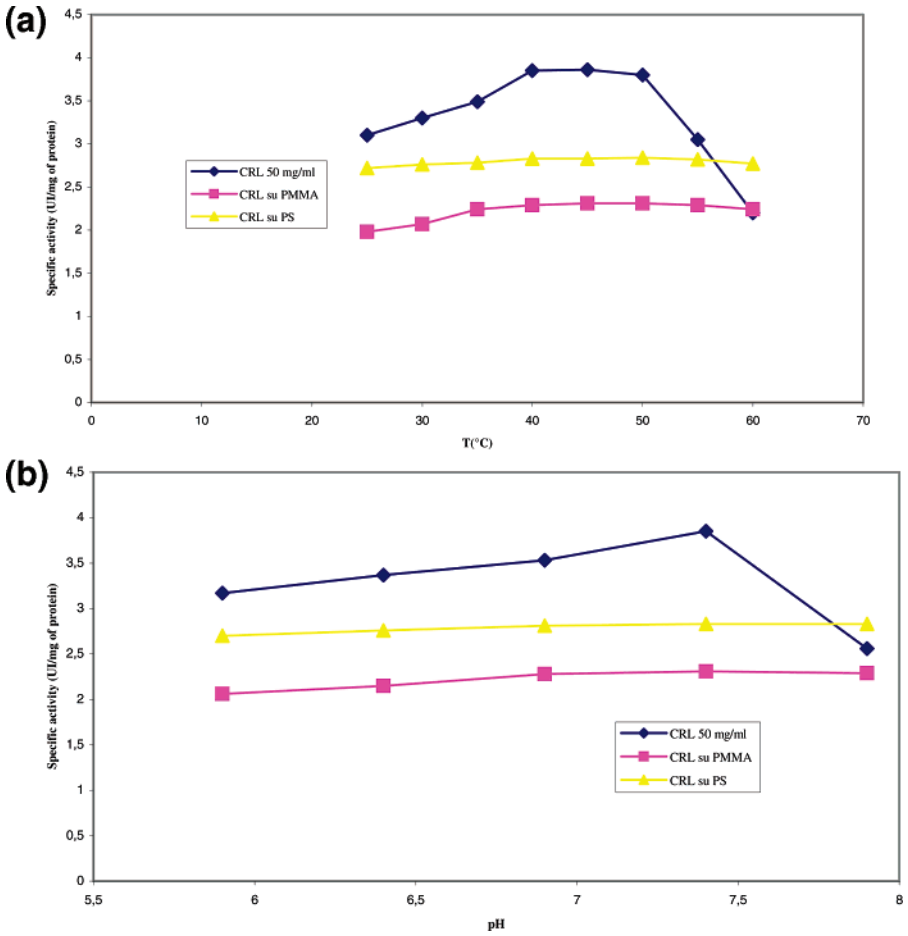

Figure 4. Thermal (a) and pH (b) stabilities of free and immobilized CRL on nanostructured PMMA and PS. Reaction conditions: 5 mg of enzyme; reaction volume, 2.5 mL; phosphate buffer, 0.1 M and 100 μL of tributyrin at pH 7.6; $T = 37$ °C; reaction time, 30 min; average diameter of nanoparticles, 300 nm.

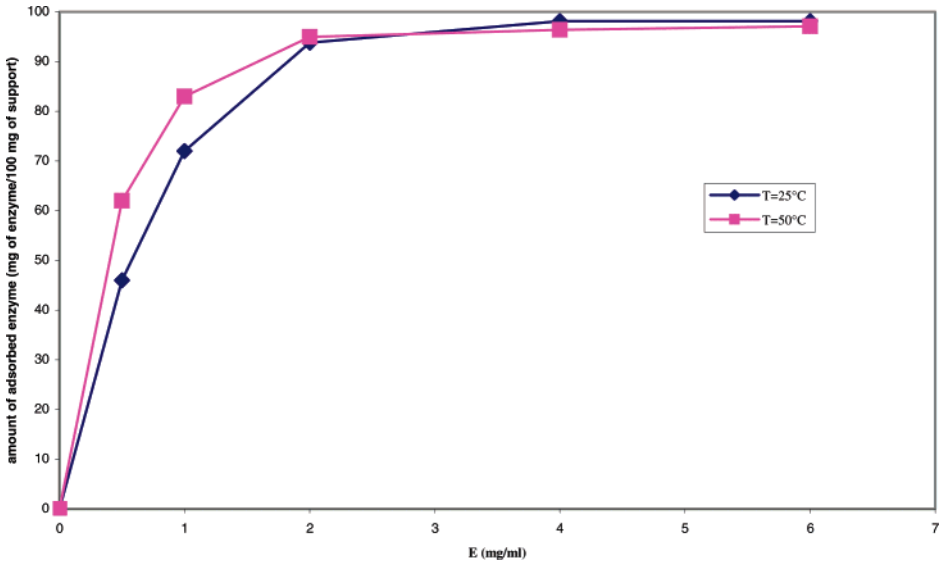

Figure 5. Adsorption isotherms of CRL immobilized on nanostructured PMMA at different temperatures. Reaction conditions: enzyme, 50 mg/mL; reaction volume, 2 mL; polymer, 100 mg; phosphate buffer, 0.1 M at pH 7.6; $T = 25$ °C; average diameter of nanoparticles, 300 nm.

agreement with the adsorption isotherms obtained for CRL and PCL reported above.

From an industrial point of view, one of the most important features concerning immobilized CRL and PCL preparations is the reuse stability, which can really reduce the process costs. In order to evaluate reuse stability, the immobilized lipases were washed in PBS after every run and reintroduced into fresh solution. This process was repeated up to 10 cycles, and no loss of activity was observed for the immobilized enzyme within the employed conditions (data not shown).

As is well known, lipases are useful biocatalysts in synthetic reactions because they can catalyze both esterifications and transesterifications in nonaqueous media. In this work, we tested the esterase activities of free and immobilized lipases in the

Table 2. Activity and Selectivity of CRL and PCL Free and Immobilized[a] on Nanostructured Polymers Employed in the Transesterification Reaction of (±)-1-Phenylethanol with Vinylacetate in Different Organic Solvents

	n-hexane $\log_p = 3.5$		*t*-buthyl-methyl-ether $\log_P = 0{,}94$		acetonitrile[b] $\log_P = 0{,}03$
enzymes	yield (%)	ee_p%	yield (%)	ee_p%	yield (%)
free CRL	9.7 ± 3%	52.73 ± 4%	21.5 ± 3%	97.51 ± 4%	<10
CRL on amorphous PMMA	13.2 ± 3%	57.45 ± 4%	23.6 ± 3%	96.40 ± 4%	<10
CRL on nanostructured PMMA	23.0 ± 3%	65.92 ± 4%	30.5 ± 3%	97.82 ± 4%	<10
CRL on amorphous PS	10.8 ± 3%	55.83 ± 4%	24.2 ± 3%	95.81 ± 4%	<10
CRL on nanostructured PS	16.7 ± 3%	68.21 ± 4%	28.7 ± 3%	97.38 ± 4%	<10
free PCL	28.5 ± 3%	100 ± 4%	14.2 ± 3%	100 ± 4%	<10
PCL on amorphous PMMA	40.2 ± 3%	99.87 ± 4%	23.6 ± 3%	100 ± 4%	<10
PCL on nanostructured PMMA	54.3 ± 3%	100 ± 4%	50.4 ± 3%	100 ± 4%	<10
PCL on amorphous PS	32.4 ± 3%	100 ± 4%	21.8 ± 3%	100 ± 4%	<10
PCL on nanostructured PS	42.0 ± 3%	100 ± 4%	43.2 ± 3%	100 ± 4%	<10

[a] Reaction conditions: enzyme, 5 mg; reaction volume, 1 mL; reaction temperature, $T = 40$ °C; reaction time, 6 h. [b] The enantiomeric excess in acetonitrile was not determined.

transesterification of (±)-1-phenylethanol with vinyl acetate. As reported in the literature,[38] the phenyl group present in (±)-1-phenylethanol activates the transesterification reaction, leading to higher reaction yields. Moreover, tautomerization of vinyl alcohol to acetaldehyde is important for it to be removed from the reaction medium, shifting the reaction equilibrium toward the synthesis of the desired products. Table 2 reports the results of esterase activity and selectivity of free and immobilized CRL and PCL preparations in the selected reaction in organic solvents. The reaction media were selected on the basis of polymer solubility and solvent log P_{ow}. The data point out that both enzymes increased their activity after adsorption as a consequence of the strong hydrophobic interactions of the protein with the carrier, which seems to prevent, to some extent, the possible surface-induced inactivation. Moreover, a remarkable feature observed for both lipases is that the activity of the adsorbed lipases onto the nanostructured polymers is substantially higher than that of free lipases in solution, with conversion values that from 10% (free CRL) rise up to 50% for the immobilized one in *n*-hexane. As far as CRL bioconjugates are concerned, the enantioselectivity also seems to be influenced by the nature of the support. A comparison of the enantiomeric excess (ee) values, for the free and bioconjugated CRL preparations, shows that there is an increase of the selectivity upon adsorption onto the polymeric nanostructured carriers. These results highlight the possibility that CRL molecules have retained their catalytic activity also in the organic medium and that it is also possible to obtain new protein conformers stabilized after adsorption on nanoparticles with improved enantioselectivity.

Conclusions

CRL and PCL commercial preparations were immobilized on nanostructured PMMA and PS by simple addition of lipase molecules to polymer suspensions. The established adsorption protocols permitted us to prepare stable bioconjugates with an activity retention up to 74%. Energy filtered-transmission electron microscopy (EF-TEM) performed on immuno-gold labeled samples revealed that the enzyme preferentially binds to the polymer nanoparticles and that the binding does not affect the nanostructured features of the carriers

After immobilization, pH, and thermal and reuse stability, together with the enantioselectivity in the resolution of a chiral alcohol for industrial, use were substantially enhanced. These results pointed out a possible lipase molecule stabilization at the interface with the polymers. The simple method described in this work clearly indicates that nanostructured synthetic and biocompatible polymers can be usefully employed in enzyme immobilization technology for industrial applications.

Further work will be done in order to study in depth the conformational structure of the enzyme molecules on the surface of nanostructured polymers and to investigate the characteristics of lipase adsorption on nanostructured polymers with different chemical structure.

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