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Journal of Molecular Catalysis B: Enzymatic 40 (2006) 30–37
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Entrusting poly(acrylonitrile-*co*-maleic acid) ultrafiltration hollow fiber membranes with biomimetic surfaces for lipase immobilization

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Received 11 November 2005; received in revised form 1 February 2006; accepted 2 February 2006
Available online 24 March 2006

Abstract

One natural biomacromolecule, gelatin, was tethered on the surface of poly(acrylonitrile-*co*-maleic acid) (PANCMA) ultrafiltration hollow fiber membrane to fabricate dual-layer biomimetic support for enzyme immobilization in the presence of 1-ethyl-3-(dimethyl-aminopropyl) carbodiimide hydrochloride (EDC)/*N*-hydroxyl succinimide (NHS). Attenuated total reflectance Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy were employed to confirm the chemical changes of the modified PANCMA membrane surfaces. Lipase from *Candida rugosa* was immobilized on this dual-layer biomimetic support using glutaraldehyde (GA), and on the nascent PANCMA membrane using EDC/NHS as coupling agent, respectively. The properties of the immobilized enzymes were assayed and compared with the free one. It was found that there was an increase of the activity retention of the immobilized lipase on the gelatin-modified membrane (49.2%), compared to that on the nascent membrane (33.9%). The kinetic parameters of the free and immobilized lipases, K_m and V_{max}, were also assayed. In comparison with the immobilized lipase on the nascent membrane, there was a decrease of the K_m value and an increase of the V_{max} value for the immobilized lipase on the gelatin-modified membrane. Results also indicated that the pH and thermal stabilities of lipases increased upon immobilization. The residual activities of the immobilized lipases were 55% on the gelatin-modified membrane and 62% on the nascent PANCMA membrane, after 10 uses.

Keywords: Poly(acrylonitrile-*co*-maleic acid); Ultrafiltration hollow fiber membrane; Lipase; Enzyme immobilization; Gelatin; Dual-layer biomimetic support; Biocompatibility

1. Introduction

As a biocatalyst, lipases have received increasing attention for biotransformation and polymer synthesis [1–5]. However, practical applications prefer immobilized enzymes since they offer easy catalyst recycling, feasible continuous operations and simple product purification. For enzyme immobilization, it is very important to choose a proper support since its interaction with enzyme may have a great influence on the stability and kinetics of the enzyme. There are various supports available for enzyme immobilization, which can be classified into three general types: inorganic particles, synthetic polymers and natural macromolecules. The natural macromolecules, such as chitosan [6,7], gelatin [8], cellulose [9,10], agarose and carrageenan [11,12], are biocompatible and non-toxic for enzyme immobilization. Nevertheless, the weak mechanical stabilities of these natural materials have limited their applications. On the contrary, the inorganic particles and synthetic polymers, for example, porous glass [13], magnetic iron oxide [14], polypropylene [15–17], polyacrylonitrile and nylon [18,19] normally possess good mechanical stability, but are relatively non-biocompatible. Therefore, it is still needed to fabricate perfect supports for enzyme immobilization.

In recent years, there is a trend to increase the biocompatibility of support for enzyme immobilization [20,21], by introducing a biofriendly interface on the support surface through surface modification technologies. It is considered as effective method and based on the facts that the lack of the biocompatible surfaces lead to rapid denaturation and sharply decrease the activity of surface-bound enzymes [22], while the biocompatible surfaces reduce some non-biospecific enzyme–support interactions, create a specific microenvironment for the enzymes and

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doi:10.1016/j.molcatb.2006.02.001

thus benefit the enzyme activity. Among various surface modification protocols such as coating, adsorption, self-assembly and graft polymerization, it is relatively easier and effective to directly tether natural macromolecules on the support surface to form a biomimetic layer for enzyme immobilization. In fact, this protocol has been used in tissue engineering recently [23,24].

In our previous work [25], a dual-layer biomimetic support had been prepared for enzyme immobilization, by tethering a natural macromolecule, chitosan, on the poly(acrylonitrile-*co*-maleic acid) (PANCMA) hollow fiber ultrafiltration membrane surface containing reactive carboxyl groups in the presence of EDC/NHS. The dual-layer biomimetic support can be defined as the support with a biomimetic layer entrusted on the support surface through surface modification technology. In this work, another natural macromolecule, gelatin, was also tethered on the PANCMA membrane surface to fabricate a novel dual-layer biomimetic support. Gelatin (a protein) derived from collagen is easily available and low in cost [26]. It shows biological properties which are almost identical with those of collagen. These features make gelatin widely used in a variety of biomedical applications. Since both chitosan and gelatin contain reactive groups (amino groups) and have been successfully used as enzyme immobilization supports, it is reasonable to choose them to form biomimetic layers on the PANCMA membrane surface for enzyme immobilization. On the other hand, chitosan and gelatin belong to two kinds of natural macromolecules, polysaccharide and protein, respectively. Therefore, it may provide a possibility to directly compare the interaction difference of polysaccharide/enzyme and protein/enzyme at similar conditions by tethering them on the same support for enzyme immobilization. Lipase, one kind of the most utility enzyme, was immobilized on these dual-layer membranes and on the nascent PANCMA membrane with glutaraldehyde and EDC/NHS as coupling agent, respectively. The effect of immobilization process on the activity, catalytic properties, pH and thermal stabilities, kinetic parameters and reusability of the enzyme was investigated.

2. Experimental

2.1. Materials

Lipase (from *Candida rugosa*), Bradford reagent, bovine serum albumin (BSA, molecular mass: 67,000 Da), 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxyl succinimide (NHS), *p*-nitrophenyl palmitate (*p*-NPP) and 2-morpholino-ethane sulfonic acid (MES) were purchased from Sigma and used as received. Gelatin was purchased from Gouyao Chemical Reagent Company Limited, China. All other chemicals are of analytical grade and used without further purification.

Poly(acrylonitrile-*co*-maleic acid) hollow fiber ultrafiltration membrane was fabricated in our laboratory according to the reported process [27–29]. The outer and the inner diameters of the hollow fiber ultrafiltration membrane are 850 μm and 545 μm, respectively, with water flux of 146 L/m^2 h atm, BSA rejection of 96%, and breaking strengthen of 135 N/cm^2. The molar fraction of maleic acid in the copolymer is 7.5%.

2.2. Preparation of low molecular weight gelatin

A sample of 7.8 g gelatin with high molecular weight (HMW gelatin with molecular mass of 160,000 Da) was stirred for 1 h and dissolved absolutely in 100 mL de-ionized water to form a solution (78 mg/mL) at 80 °C. Then, 0.6 g citric acid monohydrate was added into the solution to modulate the pH value of the solution to 3.0 and stirred for 4 h at 80 °C. After this, 0.1 M NaOH solution was added to modulate the pH value of the solution to 7.0 to terminate the hydrolysis. The product was collected with rotatory evaporator at 60 °C, washed several times with de-ionized water and dried in vacuum oven at room temperature. Following this process, the low molecular weight gelatin (LMW gelatin with and molecular mass of 8000 Da) was obtained. The M_w was characterized with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

2.3. Preparation and characterization of the dual-layer biomimetic membranes

A bundle of hollow fiber membrane was thoroughly washed with de-ionized water, and then rinsed with acetic acid buffer solution (50 mM, pH 5.0). After this the membrane was submerged into the gelatin solution (15 mg/mL in acetic acid buffer solution, 50 mM, pH 5.0) in the presence of EDC/NHS (10 mg/mL, the molar ratio of EDC to NHS = 1:1) and stirred gently for 24 h at room temperature. Finally, the modified membrane was taken out, washed several times with de-ionized water to remove free gelatin. Following this process, the gelatin-modified membrane was obtained.

In order to investigate the chemical difference between the nascent PANCMA membrane and the dual-layer biomimetic membranes and confirm the tethering of gelatin on the membrane surface, respectively, Fourier transform infrared spectroscopy (FT-IR, Vector 22) with an ATR unit (KRS-5 crystal, 45°) and X-ray photoelectron spectroscopy (PHI 5000C ESCA System) were used. Water contact angle on the membrane surface was measured with a sessile drop method at 25 °C under an atmosphere of saturated water vapor by a contact angle goniometer (Dataphysics, OCA20, Germany) equipped with video camera. At least 10 contact angles were averaged to get reliable data.

2.4. Immobilization of lipase on the gelatin-modified membranes

Schematic representatives for the preparation of the support and the enzyme immobilization are shown in Fig. 1. Lipase was immobilized onto the gelatin-modified membranes by a GA activation procedure. An appropriate amount of the membrane was submerged into the GA solution which was composed of 25% GA water solution and de-ionized water (5%, v/v) and stirred for 12 h at room temperature. The activated supports was taken out, washed several times with de-ionized water to remove excess GA, then washed with phosphate buffer solution (PBS, 50 mM,

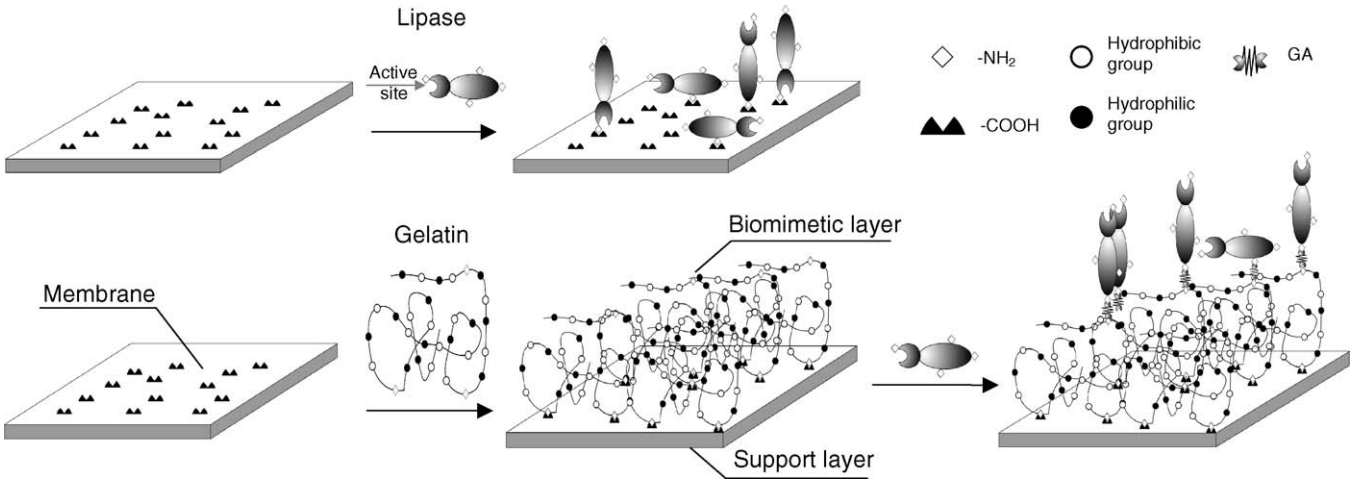

Fig. 1. Schematic representative for the preparation of the support and enzyme immobilization.

pH 6.0) and submerged into the enzyme solution (2 mg/mL in PBS, 50 mM, pH 6.0). The immobilization process was carried out at 4 °C in a shaking water bath for 3 h. After this, the membrane was taken out, thoroughly rinsed with PBS (50 mM, pH 6) and then immersed into a sodium borohydride solution (5 mg/ml) dissolved in a pH 8.0 borate buffer at 5 °C for 10 min. Finally, the membrane was taken out and thoroughly rinsed with de-ionized water.

The amount of immobilized protein on the support was determined by measuring the initial and final concentrations of protein within the enzyme solutions and washings using Coomassie Brilliant Blue reagent following Bradford's method [30]. BSA was used as standard to construct the calibration curve. The immobilization capacity of the protein on the support was defined as the amount of protein (mg) per square meter of the support. Both the outer and the inner surface area were calculated from the diameters and length of the hollow fiber membrane.

2.5. Immobilization of lipase on the nascent PANCMA membrane

Lipase was immobilized onto the nascent PANCMA membranes by an EDC/NHS activation procedure. An appropriate amount of the membrane was thoroughly washed with de-ionized water, and then rinsed with MES buffer (50 mM, pH 6.0). After this, the pretreated membranes were submerged into the EDC/NHS solution (20 mg/mL in MES buffer, 50 mM, pH 6.0, the molar ratio of EDC to NHS = 1:1) and shaken gently for 6 h at room temperature. The activated membranes were taken out, washed several times with PBS (50 mM, pH 5.5) and submerged into the enzyme solution (2 mg/mL in PBS, pH 5.5). The immobilization process was carried out at 4 °C in a shaking water bath for 1 h. Finally, the membranes were taken out, thoroughly rinsed with PBS (50 mM, pH 5.5) and then rinsed with de-ionized water. The amount of immobilized protein on the membrane was determined as described above.

2.6. Activity assay of free and immobilized lipases

The reaction rate of the free and immobilized lipase preparations was determined according to the method reported by Chiou and Wu with only minor modification [31]. In the standard conditions, the reaction mixture was composed of 1.0 mL ethanol containing 14.4 mM *p*-NPP and 1.0 mL PBS (50 mM, pH 7.5) in an Erlenmeyer flask. The reaction was started by addition of 0.10 mL free lipase preparation (or 25 mg immobilized lipase preparation). The mixture was incubated at 37 °C under reciprocal agitation at 120 strokes per minute. After 5 min of reaction, agitation was stopped, and then the reaction was terminated by adding 2.0 mL of 0.5 N Na_2CO_3 followed by centrifuging for 10 min (10,000 rpm). The supernatant of 0.50 mL was diluted 10 folds with de-ionized water, and measured at 410 nm in a UV–vis spectrophotometer (UV-1601, Shimadzu, Japan) against a blank without enzyme and treated in parallel. The reaction rate was calculated from the slope of the absorbance versus the time curve. Molar extinction coefficient of $14.5 \times 10^3\ M^{-1}\ cm^{-1}$ for *p*-nitrophenol (*p*-NP), which was determined from the absorbance of standard solutions of *p*-NP in the reaction medium, was used.

One enzyme unit was the amount of biocatalyst liberating 1.0 μmol of *p*-NP per minute in these conditions. Activity was defined as the number of lipase unit per square meter of support. Specific activity was defined as the number of enzyme unit per milligram of protein. Activity retention was defined as the ratio of the activity of the amount of the enzyme coupled on the hollow fiber membrane to the activity of the same amount of free enzyme.

2.7. pH and thermal stability measurements

The pH stabilities of the free and immobilized lipases were assayed by immersing them in PBS (50 mM) in the pH range of 3–10 for 1 h at 25 °C and then determining their activities.

The thermal stabilities of the free and immobilized lipases were assayed by immersing them in PBS (50 mM, pH 7.0) for 2 h at 50 °C and periodically determining their activities.

3. Results and discussion

3.1. Preparation of the gelatin-modified membrane

To optimize the extent of gelatin tethered, two kinds of gelatin with different molecular weight were used and the results were compared. For gelatin with molecular weight of 8000 Da, the extent of gelatin tethered was 1006 mg/m^2 membrane, while that was 45 mg/m^2 membrane for gelatin with molecular weight of 160,000 Da. These results demonstrated that decreasing the molecular weight of gelatin could significantly enhance its tethering on the membrane surface. Commonly, it is rather difficult to carry out chemical reaction directly between macromolecules due to their steric hindrances. Such hindrance is more serious for gelatin with high molecular weight than that with low molecular weight. Therefore, gelatin with molecular weight of 8000 Da was chosen and applied in next experiments.

However, it was found that the amount of natural macromolecule tethered on the PANCMA membrane surface decreased from 1162 mg/m^2 membrane for chitosan to 1006 mg/m^2 membrane for gelatin [25]. This phenomenon could be explained by the different structure between the two natural macromolecules. Chitosan, which is a polysaccharide, consists of repetitive units. In our case, the deacetylation degree of chitosan is about 84%, which means that there are a large number of amino groups that can react with the carboxyl groups on the PANCMA membrane surface to form amide bonds. On the contrary, gelatin is a kind of protein which may contain only two residual amino groups from lysine and arginine. Therefore, the fewer amino number of gelatin led to a lower tethered amount on the PANCMA membrane surface. Moreover, the molecular weight of gelatin (8000 Da), which was higher than that of chitosan (7000 Da), could cause more steric hindrance for the tethering of gelatin.

Chemical changes on the PANCMA membrane surface with the tethering of gelatin were characterized by attenuated total reflectance FT-IR (ATR/FT-IR) and X-ray photoelectron spectroscopy (XPS). The FT-IR/ATR spectra of the gelatin, the gelatin-modified membrane and the control PANCMA membrane are given in Fig. 2. It was observed that the spectrum of control PANCMA membrane (Fig. 2(c)) showed an absorbance band at 1725 cm^{-1}, which was the characteristic band for carboxyl group. Tethering gelatin on the membrane surface could be confirmed by the weakness of the absorbance band for carbonyl group at 1720 cm^{-1} (Fig. 2(b)), which was due to the conversion of carboxyl groups on the membrane surface into amide groups between gelatin and the membrane. In addition, compared with the spectrum of the nascent PANCMA membrane, there were two absorbance bands for the superposition of stretching vibration of O–H and N–H groups at 3422 cm^{-1} and for C–O group at 1030 cm^{-1}, due to the tethered gelatin.

Fig. 3 shows the XPS survey scan spectra of gelatin, the gelatin-modified membrane and the nascent PANCMA membrane, respectively. In the case of the original PANCMA membrane surface, there were three peaks at 289, 404 and 536 eV corresponding to C_{1S}, N_{1S} and O_{1S} (Fig. 3(c)). As for the gelatin-modified membrane surface, an increased O_{1S} peak intensity

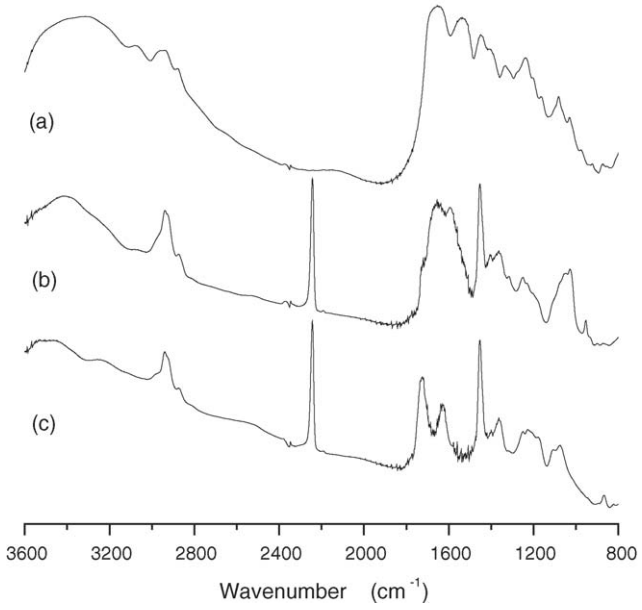

Fig. 2. FT-IR spectra of: (a) gelatin, (b) the gelatin-modified PANCMA membrane and (c) the nascent PANCMA membrane.

and decreased C_{1S} and N_{1S} peak intensities were found upon the introduction of gelatin on the membrane surface (Fig. 3(b)). The elemental compositions on the surface of gelatin, the gelatin-modified membrane and the nascent PANCMA membrane, calculated from the XPS spectra, are compiled in Table 1. Results indicated that the oxygen content of the gelatin-modified membrane surface was increased from 8.44% to 13.56% due to the immobilization of gelatin with a high O/C ratio, whereas the carbon and nitrogen content decreased from 75.74% and 15.82% to 71.20% and 15.07%, respectively. Furthermore, element sulfur (0.17%), which belonged to gelatin (0.67%), appeared on the membrane surface after the modification. These data confirmed the occurrence of the tethering of gelatin on the PANCMA membrane surface.

Additionally, the water contact angle decreased from $45.2 \pm 0.3^\circ$ for the nascent PANCMA membrane to $30.5 \pm 0.3^\circ$ for the gelatin-modified membrane. These results demonstrated that the hydrophilicity of the PANCMA membrane was improved by the tethering of gelatin onto the membrane surface.

3.2. Immobilization of lipase

The activities of the free and immobilized enzymes under optimum reaction conditions (pH and temperature) are compared in Table 2. The amount of bound protein and the activity were 53.7 ± 1.4 mg/g and 767 ± 64 U/m^2 on the nascent PANCMA membrane, 66.5 ± 1.8 mg/m^2 and 1243 ± 87 U/m^2 on the chitosan-modified membrane [25], and 54.6 ± 1.5 mg/m^2 and 1130 ± 72 U/m^2 on the gelatin-modified membrane, respectively. In comparison with the free enzyme, the immobilized enzymes under their optimum reaction conditions retained 33.9% of the activity on the nascent PANCMA membrane, 44.5% on the chitosan-modified membrane and 49.2% on the gelatin-modified membrane.

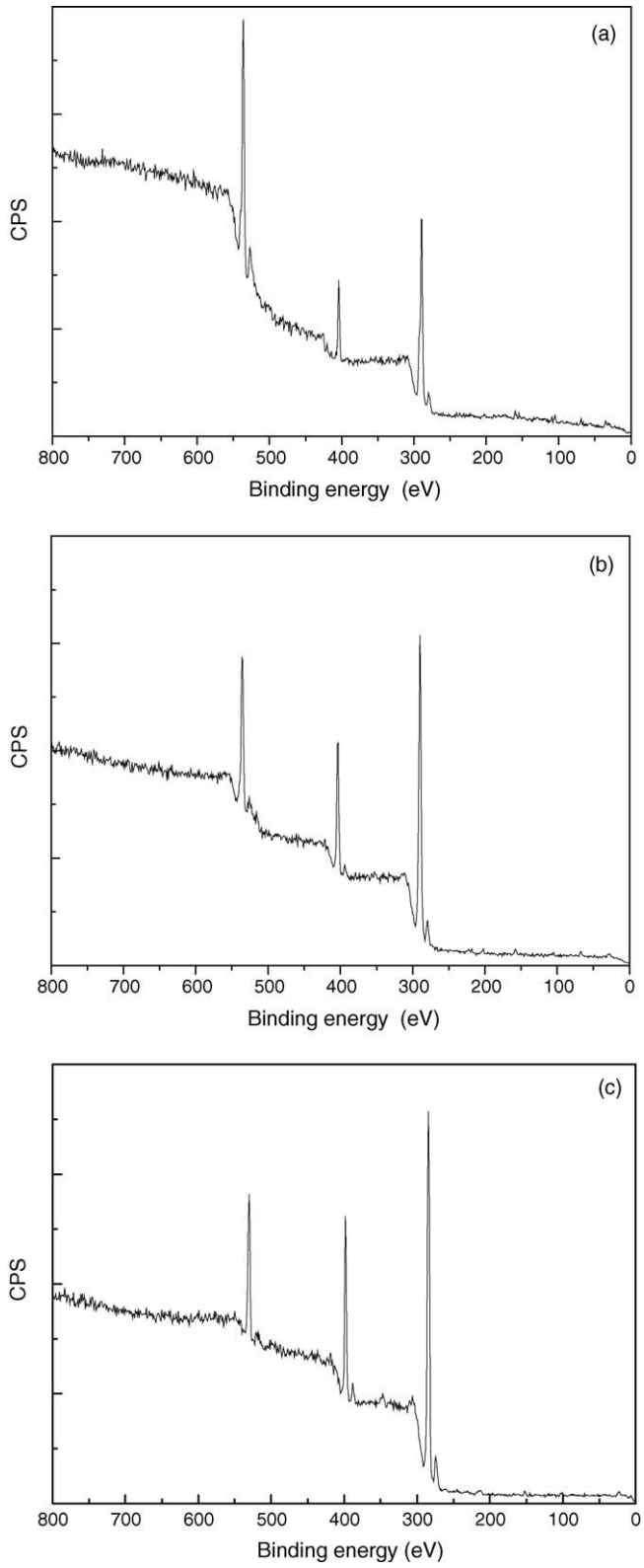

Fig. 3. XPS spectra of: (a) gelatin, (b) the gelatin-modified PANCMA membrane and (c) the nascent PANCMA membrane.

Commonly, there is a decrease of enzyme activity after its immobilization. This is attributed to the minor modification in the enzyme three-dimensional structure that may lead to the distortion of amino acid residues involved in catalysis, the presence of random immobilization which causes the analytic approach to the active site of the enzyme hindered, and the limitations imposed by the slow mass transfer of substrate or product to/from the active site of the enzyme. In our previous work [25], there was an increase of the activity retention of the immobilized enzyme from 33.9% on the nascent PANCMA membrane to 44.5% on the chitosan-modified membrane. Several reasons could explain this phenomenon. Firstly, the chitosan-layer on the support surface could create a biocompatible microenvironment for the immobilized lipase, since chitosan as one natural macromolecule possesses excellent biocompatibility and hydrophilicity which usually lead to high activity retention of immobilized enzyme on them. Secondly, the immobilization of lipase on the hydrophilic chitosan-layer might benefit the exposition of its active site [31] and the decrease of random coupling between lipase and the support, due to the special structure of lipase, hydrophobic region surrounding its active site [32]. Finally, the use of GA which introduced a penta-carbon chain might be in favor of the contact of lipase with substrate.

Table 1
Element composition of the gelatin and the membranes

Sample	Elemental compositions			
	C (%)	N (%)	O (%)	S (%)
Gelatin	59.81	13.57	25.95	0.67
Gelatin-modified PANCMA membrane	71.20	15.07	13.56	0.17
Nascent PANCMA membrane	75.74	15.82	8.44	0

In this work, similar to the case of the chitosan-modified membrane, the immobilized enzyme on the gelatin-modified membrane showed high activity retention (49.2%) and the same reasons could explain this result. Interestingly, however, it appeared that the activity retention of the immobilized enzyme on the gelatin-modified membrane was higher than that on the chitosan-modified membrane due to two possible factors. On one hand, compared to gelatin, there are a large number of amino groups in chitosan, which are potential reaction sites for covalent coupling with enzyme. Thus, it became easier to immobilize one enzyme molecule through multiple point chemical bonds on the chitosan-modified membrane, which substantially reduced its enzymatic activity [33,34]. On the other hand, after the two natural macromolecules were tethered, the properties of the membrane surfaces were changed since the different biomimetic layers formed. Therefore, the interactions between the immobilized enzyme and the membrane surfaces were also changed and the gelatin layer might create a more favor microenvironment for the immobilized lipase to retain its activity, in comparison with the chitosan layer.

3.3. Kinetic parameters

Table 3 lists the kinetic parameters K_m and V_{max} from double reciprocal plot. It was found that the K_m values were 0.45 mM, 1.36 mM, 1.43 mM and 1.15 mM for the free, the nascent PANCMA membrane bound, the chitosan-modified membrane bound and the gelatin-modified membrane bound enzymes,

Table 2
Activity of the free and immobilized lipases under optimum reaction conditions

Sample	Temperature (°C)	pH	Activity (U/m^2)	Bound protein (mg/m^2)	Specific activity (U/mg protein)	Activity retention (%)
Free lipase	37	7.5	–	–	42.1	100
Lipase immobilized on the nascent membrane	45	7.5	767 ± 64	53.7 ± 1.4	14.3	33.9
Lipase immobilized on the chitosan-modified membrane	45	7.0	1243 ± 87	66.5 ± 1.8	18.7	44.5
Lipase immobilized on the gelatin-modified membrane	43	7.5	1130 ± 72	54.6 ± 1.5	20.7	49.2

Table 3
Activity and kinetic parameters of the free and immobilized lipases

Sample	V_{max} (U/mg)	K_m (mM)	Specific activity (U/mg)	Activity retention (%)
Free lipase	46.4 ± 3.2	0.45 ± 0.04	42.1	100
Lipase immobilized on the nascent PANCMA membrane	16.1 ± 1.2	1.36 ± 1.10	14.3	33.9
Lipase immobilized on the chitosan-modified membrane	21.2 ± 1.7	1.43 ± 0.10	18.7	44.5
Lipase immobilized on the gelatin-modified membrane	23.2 ± 1.7	1.15 ± 0.92	20.7	49.2

respectively. There was an obvious increase in the K_m values for these immobilized enzymes. The increase in the K_m values was either due to the conformational changes of the enzyme resulting in a lower possibility to form substrate–enzyme complex or to the lower accessibility of the substrate to the active site of the immobilized enzyme caused by the increased diffusion limitation.

Interestingly, the K_m value of the immobilized lipase on the gelatin-modified membrane was lower than that on the chitosan-modified membrane [25]. There were two reasons to explain this result. Firstly, compared to the chitosan-modified membrane bound enzyme, the immobilized enzyme on the gelatin-modified membrane which was coupled by less multiple chemical bonds could easily convert its conformation to form substrate–enzyme complex. Secondly, some properties of the gelatin layer might lead to the lower K_m value. As mentioned above, gelatin is a protein and is composed of the same amino acids as collagen [26]. It can be concluded from the composition of gelatin that $–CH_3$, $–CH_2–$, $–NH_2$, –NH–, –OH, –SH, $–SCH_3$ and –COOH groups are present in the gelatin layer. These groups can be classified into two general types: hydrophilic groups and hydrophobic groups. The presence of the hydrophobic groups in the gelatin layer could benefit the adsorption of hydrophobic substrate molecules on the membrane surface to close the active site of enzyme through hydrophobic interaction, and hence influenced the kinetic parameter K_m. Moreover, as reported by other researchers [20,35], the moderate hydrophobicity–hydrophilicity of support surface can benefit the activity improvement of lipase. There could be some interactions formed between the immobilized enzyme and the gelatin layer with the hydrophilic and hydrophobic groups, which might help the immobilized enzyme to take a favor conformation to couple the substrate molecule.

As shown in Table 3, the V_{max} values of enzymes demonstrated a decrease upon immobilization from 46.4 U/mg for the free lipase to 16.1 U/mg, 21.2 U/mg and 23.2 U/mg for the immobilized lipases on the nascent PANCMA membrane, the chitosan-modified membrane and the gelatin-modified membrane, respectively. It was found that the V_{max} value of the immobilized lipase on the nascent membrane was lower than that on the dual-layer biomimetic supports. This was attributed to the biocompatible microenvironment for the immobilized lipase created by the biomimetic-layer on the support surface. Furthermore, the V_{max} value of the immobilized lipase on the gelatin-modified membrane was higher than that on the chitosan-modified membrane, which corresponded to the activity retention of the immobilized lipase on these supports and could be explained by the same reasons.

3.4. pH and thermal stabilities

Fig. 4(a) shows the pH stabilities of the free and immobilized lipases on these supports. There was no activity loss for the free lipase in the pH range from 4 to 6, while it was found to be stable up a pH value of 7 for the immobilized lipases. These data indicated that the pH stability of lipase could be enhanced by the immobilization process.

The thermal stabilities of the free and immobilized lipases are given in Fig. 4(b). It can be seen that the free lipase lost its initial activity within around 100 min, while the immobilized lipases retained their initial activity of about 62% for the nascent PANCMA membrane and 66% for the gelatin-modified membrane after a 120 min of heat treatment, respectively. These results indicated that the thermal stability of immobilized lipases was much better than that of the free one due to the formation of covalent bond between the enzyme and the supports, which prevented the conformation transition of the enzyme at high temperature.

3.5. Reuse stability of the immobilized lipase

One of the most important aims of enzyme immobilization for practical applications is to enhance the enzyme reuse stability. To evaluate the reuse stability, the lipase-immobilized membranes were washed with PBS (50 mM, pH 7.5) after any run and reintroduced into a fresh solution, this being repeated up to 10 cycles.

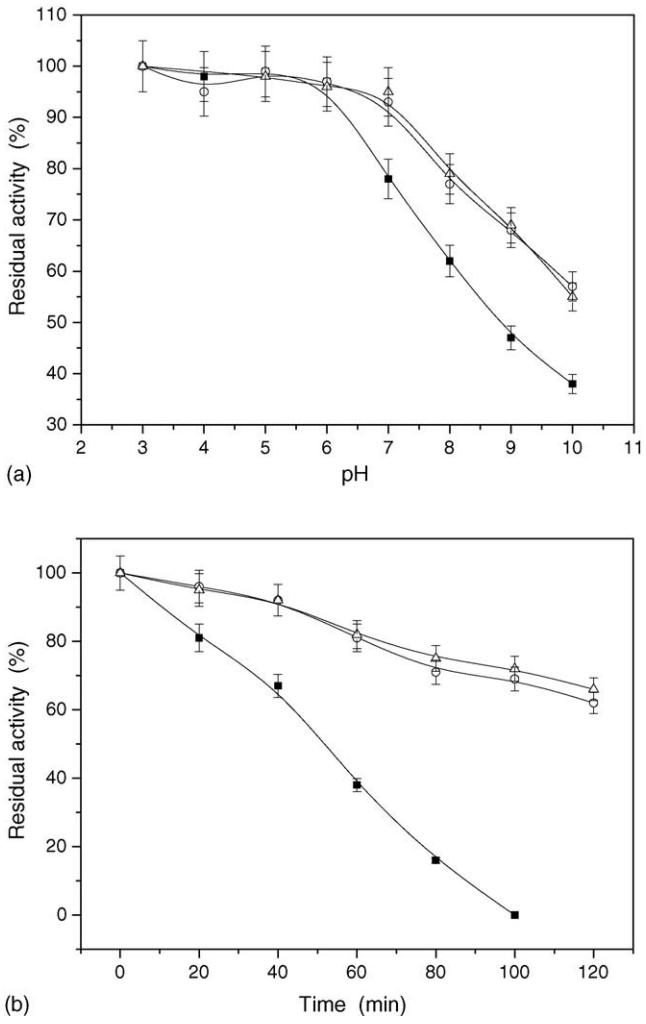

Fig. 4. pH and thermal stability of the free and immobilized lipases: the free (■), on the nascent PANCMA membrane (◯) and on the gelatin-modified PANCMA membrane (△).

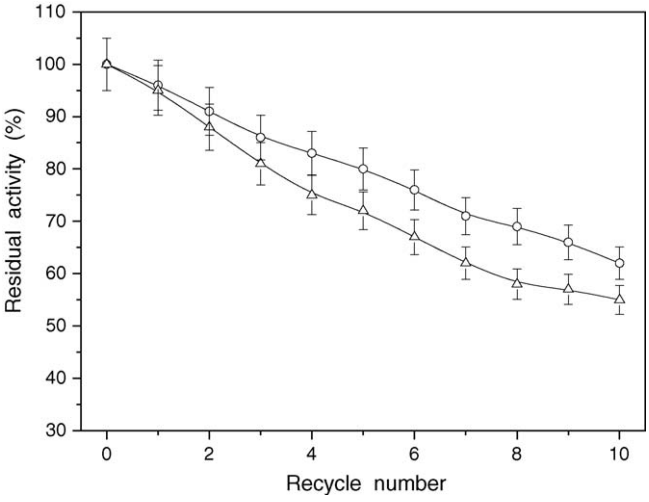

Fig. 5. Reuse stability of the immobilized lipases: on the nascent PANCMA membrane (◯) and on the gelatin-modified PANCMA membrane (△).

Fig. 5 shows the effect of repeated use on the activities of these immobilized enzymes. After 10 reuses, the residual activities of the immobilized enzymes were 62% for the nascent PANCMA membrane and 55% for the gelatin-modified membrane, respectively. These results were due to the inactivation of the enzyme caused by the denaturation of protein and the leakage of protein from the supports upon use.

4. Conclusion

In this work, one natural biomacromolecule, gelatin, was tethered on the surface of poly(acrylonitrile-*co*-maleic acid) hollow fiber ultrafiltration membrane to prepare dual-layer biomimetic support for enzyme immobilization. The results were compared with those of chitosan as the biomacromolecule. This protocol established such environments in which protein and polysaccharide could contact with enzyme at similar conditions. It was found that there was an increase of the activity retention of the immobilized lipase on the gelatin-modified membrane (49.2%) and on the chitosan-modified membrane (44.5%), compared to that on the nascent membrane (33.9%). The kinetic parameters of the free and immobilized lipases, K_m and V_{max}, were assayed. In comparison with the chitosan-modified membrane, there was a decrease of the K_m value and an increase of the V_{max} value for the immobilized enzyme on the gelatin-modified membrane. After immobilization, the pH, thermal and reuse stabilities of the immobilized enzyme increased. It is meaningful to study the interactions of polysaccharide/enzyme and protein/enzyme and to compare their difference. However, it is relatively difficult to fully understand the interactions between the two natural biomacromolecules and enzyme at this moment, since the structures of these biomacromolecules and enzyme are complicate and their function mechanism is not clearly known after immobilization. Thus, further work is still needed and this will be reported in due course.

Acknowledgements

The authors are grateful to the National Natural Science Foundation of China (Grant no. 50273032) and Programme Sino-Français de Recherches Avancées (Grant no. PRA E03-04) for financial support.

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