Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 80 (9) 1113–1125 (2015)
JSCS–4784
UDC 661.183.8+577.115:541.183
Original scientific paper

Adsorption of *Candida rugosa* lipase onto alumina: effect of surface charge

LIDIJA T. IZRAEL ŽIVKOVIĆ[1]*#, LJILJANA S. ŽIVKOVIĆ[2#], BOJAN M. JOKIĆ[3], ANDRIJA B. SAVIĆ[2] and IVANKA M. KARADŽIĆ[1]

[1]*University of Belgrade, School of Medicine, Institute of Chemistry in Medicine, Visegradska 26, 11000 Belgrade, Serbia,* [2]*University of Belgrade, The Vinča Institute of Nuclear Sciences, 11000 Belgrade, Serbia and* [3]*University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11001 Belgrade, Serbia*

(Received 22 February, revised 16 April, accepted 30 April 2015)

Abstract: The impact of the surface charge of alumina supports on the adsorption of *Candida rugosa* lipase was investigated in terms of the zeta potentials of the adsorption partners. The lipase adhered onto alumina with similar efficiency under both repulsive and attractive electrostatic conditions, shifting the zeta potential of the support towards that of the enzyme. The behavior was explained by a heterogeneous distribution of the surface charge of the lipase molecule. Special emphasis in this study was placed on the effect of immobilization on the enzyme kinetics and principal reasons for enzyme immobilization: improvement in stability and potential for reuse. The enzyme affinity was not altered by its adsorption onto alumina, while the V_{max} value of the lipase decreased. The thermostability of the adsorbed lipase was improved. A significant potential for reuse was found.

Keywords: alumina; lipase; adsorption; immobilization; *Candida rugosa*.

INTRODUCTION

Increasing environmental concerns have led to the replacement of conventional chemical methods with enzyme-based strategies. The utilization of enzymes as biocatalysts has become an integral part of different processes in the oil, pharmaceutical, detergent, and food industries. During the last decade, the potential of different techniques for immobilization has been studied in order to overcome instability, enhance enzyme reuse, as well as easier separation.[1] Among them, the most commonly used methods for enzyme immobilization are cross-linking or covalent binding, entrapment and adsorption. One of the simplest methods, with high commercial potential and wide applicability, is adsorption.[2] Adsorption

* Corresponding author. E-mail: lidija.izrael-zivkovic@mfub.bg.ac.rs
# These authors contributed equally to this article.
doi: 10.2298/JSC150222035I

causes little or no conformational changes of the enzyme but the linkages established between the enzyme and support are weak, so the enzyme could be easily desorbed (leaking of the enzyme from the support). The frequently used supports are inert polymers and inorganic materials.[3] Alumina exhibits high mechanical strength, excellent corrosion and wear resistance and good biocompatibility[4] and it is among the mostly used ceramics.[5]

Lipases are an important group of biocatalysts as they improve a variety of reactions with high specificity and selectivity.[6,7] *Candida rugosa* lipase has been widely used in stereoselective synthesis of pharmaceuticals, production of carbohydrate esters of fatty acids, biodiesel, biosensors, food and flavor making.[8,9] Considering the fact that *C. rugosa* is one of the most important enzymes in biotransformations, different protocols for its immobilization have been developed but novel and efficient methods are still required. Even though common and simple for performing, adsorption is a very complex phenomenon.[10]

Research teams have identified numerous factors that strongly influence enzyme loading and biocatalyst activity, such as support surface area, pore size distribution, pore volume, particle size, ionic strength as well as surface charge of both support and enzyme.[11] In a few studies, electrokinetic measurements were conducted, yielding different results. Although there is no doubt that electrostatic interactions influence the adsorption process, some authors claim that the maximal affinity of a protein towards a support surface occurs when the adsorption partners exhibit opposite surface charges,[11,12] while others suggest the highest adsorption efficiency under repulsive electrostatic conditions (same surface charge),[13,14] or at pHs close to the isoelectric point of the protein,[15] thus creating contradictory approaches.

In this regard, a previous study dealt with effect of the surface charge on the adsorption of *C. rugosa* lipase. Two inorganic supports, mesoporous silica SBA--15 and macroporous zirconia were used.[16] The electrostatic nature of the process was estimated based on the electrokinetic (zeta) potentials of the interacting partners. However, no direct correlation between adsorption efficiency and immobilization pH was hitherto established; the enzyme adhered similarly under both attractive and repulsive electrostatic conditions. Aimed at obtaining better insight into the impact of electrostatics on the adsorption of enzymes onto supports, the idea of the current study was to minimize the influence of the support morphology. For this purpose, nonporous submicron-sized alumina was selected as the model support. The same methodology was used, *i.e.*, changes in zeta (ζ) potential of the adsorption partners were monitored at different pH values and correlated with the amount of adsorbed lipase. The second part of the study focused on the effect of immobilization on the enzyme kinetics and the principal reasons for enzyme immobilization: improvement in stability and potential for reuse.

EXPERIMENTAL

Materials

Lipase from *C. rugosa* (lyophilized powder, Type VII, nominal activity 746 U mg^{-1}) and *p*-nitrophenyl palmitate (*p*-NPP) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The support material, alumina (Al_2O_3, AKP-30 Sumitomo, Japan), was a high purity (>99.99 %) α-phase, with an average particle size of 0.32 μm (size distribution: d_{90}, d_{50} and d_{10} of 0.61, 0.35 and 0.2 μm, respectively, Sedigraph 5100, Micrometrics)[17] and a specific surface area, Sp = 7 m^2 g^{-1}. All other chemicals were of analytical grade.

Enzyme assay

The activity of free lipase was measured spectrophotometrically using an assay based on the hydrolysis of *p*-NPP. The *p*-NPP solution was prepared as follows: 30 mg of *p*-NPP in 10 mL of 2-propanol was added to 90 mL of 0.05 M phosphate buffer (pH 8.0) supplemented with 200 mg of Na deoxycholate and 100 mg of gum arabic, (the final *p*-NPP concentration was 0.8 mM). The absorbance was measured at 410 nm for the first 3 min of the reaction at 25 °C. One unit (1 U) was defined as the quantity of enzyme that under the test conditions liberated 1 μmol of *p*-nitrophenol per min (ε = 18.5 mM^{-1} cm^{-1}). The reaction mixture was composed of 900 μL of *p*-NPP solution and 100 μl of lipase solution (lipase concentration 0.05 mg mL^{-1}).[18]

Activity of immobilized lipase was determined using 10 mg of immobilized preparation supplemented with 2 mL of *p*-NPP solution (the *p*-NPP of concentration was 0.8 mM, in phosphate buffer of pH 8.0) The reaction was performed at 25 °C for 3 min when it was stopped by the addition of 0.5 M sodium carbonate solution. The precipitate was removed by centrifugation at 10,000 rpm for 10 min. The supernatant was diluted 10-fold with deionized water and the absorbance was measured at 410 nm. One unit of enzyme activity (1 U) was defined as explained in the previous paragraph. The activity of the immobilized enzyme was calculated as U per g of support material.

Lipase immobilization

The effect of pH on the adsorption of lipase was investigated in the pH range 5 to 9, using the following 10 mM buffer solutions: acetate buffer (pH 5.0), phosphate buffer (pH 6.0, 7.0 and 8.0) and Tris-buffer (pH 7.6 and 9.0).

Alumina was prepared for immobilization as follows: 8 mg support mL^{-1} in the selected buffer was de-agglomerated for 10 min by sonication. Then, a lipase solution (2 mg mL^{-1} in the selected buffer) was added, the resulting suspension sonicated for 10 min and immobilization realized at room temperature under mild stirring (90 rpm). After 60 min, the immobilized lipase was precipitated by centrifugation at 10,000 rpm for 10 min using a microcentrifuge (Denver Instruments, USA). The immobilized enzyme was washed twice to remove the excess of unbound enzyme, dried for 1 h at room temperature and used for the assay.

The efficiency of immobilization was evaluated as the percent of the lipase adsorbed, which was calculated as:

$$\text{Lipase adsorbed (\%)} = \frac{100P_1}{P_0} \quad (1)$$

where P_0 is the initial concentration of lipase and P_1 is the concentration of immobilized lipase. The concentration of immobilized lipase was calculated as a difference between initial lipase concentration and lipase concentration in the supernatant after immobilization.[19]

The concentration of lipase was determined by the Bradford method using bovine serum albumin as the standard.[20]

Zeta potential measurements

Electrokinetic (zeta) potential measurements were performed by means of a Zetasizer Nano ZS instrument (Malvern, UK). The instrument uses the electrophoretic light scattering and laser Doppler velocimetry methods for determination of particle velocity, and from it, the zeta (*ζ*) potential. The integrated software calculates the *ζ*-potential from mobility values by the Henry equation, using the Smoluchowski approximation.[21,22] The measurements were performed at least in triplicate and the average values are presented. Each measurement comprised of minimum 10 runs. Only the results that met quality criteria were taken into account.

Aimed at determining of the isoelectric point (IEP) of support, the *ζ*-potential of the alumina particles was measured over a wide range of pH values (4–10), using aqueous solution of NaCl (0.01 M) as the inert background electrolyte.[23] Prior to titration, powder dispersion (1 mg mL^{-1}) was equilibrated for 24 h under mild shaking at room temperature. The *ζ*-potential measurements of lipase and that of the support particles (before and after enzyme adsorption) were then performed in the selected buffers adjusted to different pH values (5–9).

Field emission scanning electron microscopy (FESEM)

Field emission scanning electron microscopy (FESEM), using a Tescan Mira3 XMU (Czech Republic) at 20 kV, was employed to study the morphology of the support before and after the adsorption of lipase. Prior to analysis, the samples were coated with Au–Pd alloy using a sputter coater.

Determination of the kinetic parameters

Michaelis–Menten kinetics were used to describe the dependence of enzyme activity on substrate concentration for free and immobilized lipase. The kinetic parameters, V_{max} and K_m were determined for free and adsorbed lipase using solutions of *p*-NPP of the following concentrations: 0.1, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 mM, according to the above-described methods for the determination of lipase activity.

V_{max} – units of activity per milligram of protein (U mg^{-1}) and K_m – substrate concentration that gives a reaction rate of $V_{max}/2$ (mM) were estimated from the experimental data using the Lineweaver–Burk equation:

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}}\left(\frac{1}{[S]}\right) \tag{2}$$

The *y*-axis intercept is $1/V_{max}$ and the slope is K_m/V_{max}.

Thermal stability

Reaction mixtures containing free or immobilized enzyme were incubated at 50 and 60 °C for various periods (20–120 min) and quickly cooled. Enzyme activity was measured according to the standard protocol for free and immobilized enzyme with *p*-NPP as the substrate. The half-life ($t_{1/2}$) of the enzyme with k_d as the decay constant was calculated as:

$$t_{1/2} = (\ln 2)/k_d \tag{3}$$

Reuse stability

The potential for reuse was determined using 25 mg of immobilized enzyme packed in a small filter paper bag soaked into 3 mL of *p*-NPP solution for 30 min at room temperature,

under mild stirring. After each batch reaction, the immobilized lipase was washed three times with deionized water and then reused. Into 2 mL of removed supernatant solution, 2 mL of 0.5 M sodium carbonate was added to stop the reaction. The absorbance was measured at 410 nm after centrifugation at 10,000 rpm for 10 min and 10-fold dilutions.

RESULTS AND DISCUSSION

Zeta (ζ) potential and immobilization pH study

There are close analogies between protein adsorption and particle deposition[24,25] both being governed by short-range interactions between charged surfaces at the solid–liquid interface. The DLVO (Derjuangin–Landau–Verbey––Overbeek) theory[26] plays an essential role in the quantification of the processes. Although based on simple additivity of electrostatic and van der Waals forces, it is successful in predicting the basic features of colloidal stability and adsorption or deposition processes. Since the electrostatic forces are dominant for attachment, the relative charges of the surfaces dictate whether the interaction is repulsive or attractive. The isoelectric points (*IEP*) of the partners indicate the pH where the ζ potential equals zero and the range over which the interactions are favored. Thus, a prediction of the process requires knowledge of the *IEP* values for both materials.

The reactions occurring at the solid–liquid interface were the subject of numerous experimental and theoretical investigations. To better understand the adsorption of proteins onto the surface of a support (*e.g.*, the mechanism of binding, the build-up of layers, *etc.*), the electrokinetic ζ potential changes *vs.* pH were often collected.[22,27–29] However, the aim of this study was not to elucidate the mechanism of *C. rugosa* lipase adsorption onto alumina surface by means of electrokinetic measurements, but to test the zeta potential of the adsorption partners as a diagnostic tool for the efficiency of binding of the protein to the support.

The variation in zeta potential of alumina, used as support, as a function of pH is presented in Fig. 1. As is evident, the isoelectric point was located at pH

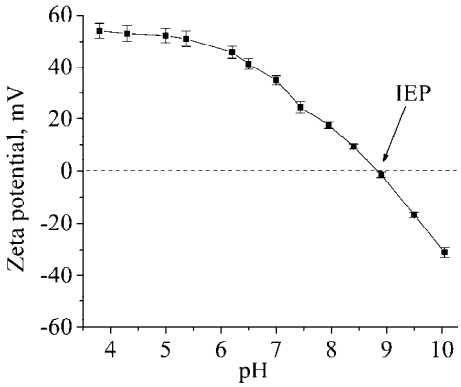

Fig. 1. Titration curve: zeta potential *vs.* pH for alumina powder in NaCl (0.01 M).

8.8, in good accordance with the literature.[17] Thus, the alumina develops positive and negative surface charges below and above this pH by protonation–deprotonation of the hydroxide groups present on the surface.[30]

Lipase from *C. rugosa* is a globular glycoprotein with molecular mass of 57 kDa. Its basic structural characteristics are summarized in Table I. With 31 acidic and 18 basic amino acids exposed on the surface,[16] its IEP is located at pH 4.65. Above this pH, the lipase acquires a negative charge and hence the enzyme should always be negative in this study.

TABLE I. Protein parameters of *C. rugosa* lipase, P20261 (UniprotKB)

Parameter	Value
Molecular mass, kDa	57
Dimensions, mm	5×4.2×3.3
Isoelectric point (IEP)	4.65
Total number of amino acids	534
Number of acidic amino acids	52
Number of basic amino acids	39
Non-polar surface, %	63
Surface acidic amino acids	31
Surface basic amino acids	18
α-Helical structure, %	38
β-Sheet structure, %	18
Post-translational modifications, PTM	Disulfide bonds: 75–112, 283–292 Glycosilation (*N*-acetyl-D-glucosamine): 329, 366
E.C. (Brenda)	3.1.1.3.1139.
3D structure in PDB	1CRL

To evaluate the effect of the surface charge of the adsorption partners on the adsorption efficiency, experiments were performed below and around the *IEP* of alumina (pH 8.8), where 2 different electrostatic scenarios were expected. The first one (pH < $IEP_{alumina}$) includes favorable adsorption conditions, *i.e.*, negatively charged lipase in the presence of positively charged alumina particles. The second adsorption scenario (pH ≈ $IEP_{alumina}$) is related to the interaction of negatively charged lipase with uncharged alumina, thus being much less favorable.

Different buffers were used over the investigated pH range 5–9. The ζ-potentials of enzyme and support, before and after adsorption, were monitored in parallel, and the percent of adsorbed lipase (η_{enz}) related to the values of the ζ-potential are summarized in Table II.

Indeed, in agreement with theoretical calculations, the lipase was negatively charged in the investigated pH range, reaching the highest absolute magnitude of ζ-potential at pH values 7–9. On the other hand, the alumina particles were positive in the acetic buffer adjusted to pH 5, as expected (Fig. 1). Surprisingly, in the presence of phosphate buffer, a charge reversal occurred: the alumina

turned highly negative even at pH 6. This result was confirmed for pH values 7–8, indicating specific adsorption of phosphates onto alumina surface occurred. A significant shift in the *IEP* towards acidic pH upon addition of PO_4^{3-} was also reported by Zheng.[31] To avoid preferential adsorption of phosphates, tris buffer was introduced, in which alumina behaved analogously as in NaCl. Likewise, at pH 7.6, the particles developed a positive charge, whilst at pH 9, a negative ζ-value was recorded.

TABLE II. ζ-potentials of free lipase and alumina, before and after adsorption of lipase, and the amount of lipase adsorbed at pH values 5–9

Buffer	ζ-potential, mV			η_{enz} / %
	Lipase	Alumina	Alumina–lipase	
pH 5 (acetic)	–4.3±0.5	+40.2±1.6	–9.4±1.1	30
pH 6 (phosphate)	–9.8±1.2	–35.9±2.1	–13.7±1.4	32
pH 7 (phosphate)	–12.4±0.4	–36.7±0.4	–25.0±2.3	33
pH 8 (phosphate)	–14.3±1.3	–30.7±1.3	–17.3±1.4	30
pH 7.6 (tris)	–14.3±0.6	+22.1±0.9	–25.4±2.2	38
pH 9 (tris)	–16.7±0.8	–12±0.5	–16.4±0.8	5

Albeit two electrostatic scenarios were expected, the phosphate buffer actually created the third repulsive electrostatic conditions, in which both alumina and lipase carried a negative surface charge.

As presented in Table II, the percent of adsorbed lipase was comparable under both attractive and repulsive electrostatic conditions. Whatever the charges of the immobilization partners were (like or dislike, as a function of the buffer used), the amount of adsorbed enzyme was moderate (around 30 %). Contrarily, at pH 9 (*i.e.*, when the pH ≈ IEP of alumina), only a small percentage (5 %) of adsorbed lipase was registered. This result is logical, since a pH value in the close vicinity of the IEP of alumina, when the particles carry only a negligible surface charge, strongly promoted their aggregation and sedimentation. Such adsorption condition was inconvenient for the lipase to reach the surfaces of the particles and adhere. On the contrary, the alumina particles were quite well dispersed at the other investigated pH values. A ζ-potential of *ca*. 30 mV (positive or negative) is normally required to achieve a reasonably stable dispersion.[30]

An anomalous adsorption of lipase molecules onto alumina bearing the same surface charge contradicts the classical DLVO theory. However, adsorption–deposition of protein–particles under repulsive electrostatic conditions is not an unusual phenomenon.[13,32,33] Although the overall charge of the lipase was negative in the selected pH range, the enzyme surface consists of positively (Lys, Arg, His) or negatively (Glu, Asp) charged, and non-polar residues dispensed into a patchwork which leads to localized interactions (Table I). The hetero-

geneous surface charge of an enzyme always establishes attractive electrostatic conditions, whatever is the charge on the support particles.

Therefore, the adsorption efficiency of enzyme depends on the dispersion state of support particles, *i.e.*, on the magnitude of the ζ-potential, not its sign, increasing enzyme availability to approach and adhere onto the surfaces of the particle. The presented results suggest that the zeta potential is a useful indicator of an adsorption process. An obvious change in the ζ-potential of the support was always registered after enzyme immobilization, indicating that the surfaces of the alumina particle were altered. Negatively charged lipase shifted the ζ-potential of support towards negative values, sometimes even more negative than its own.

The highest adsorption was achieved in the tris buffer of pH 7.6, therefore this buffer was chosen as suitable for lipase immobilization. Lipolytic activity of immobilized enzyme in chosen system was determined to be 58 U g^{-1} of alumina.

Field emission scanning electron microscopy (FESEM)

Adsorption of lipase onto support was also characterized by FESEM (Fig. 2). The powder was composed of differently sized sub-micrometer particles of irregular shapes. Their surfaces appeared smooth and nonporous, corroborating well the small value of specific surface area (7 m^2 g^{-1}). At high magnification (inset), a certain number of small, differently sized piles (20–80 nm), can be randomly spotted on the particle surfaces. They could be associated with lipase aggregates, composed of several lipase molecules (5 nm×4.2 nm×3.3 nm).

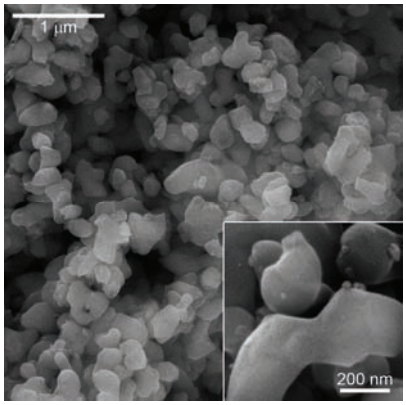

Fig. 2. FESEM micrographs of alumina before and after (inset) lipase adsorption.

The rather moderate adsorption of enzyme achieved (*ca*. 30 %) could be related to the poorly developed surface geometry of the alumina particles, not favorable for lipase adhesion. A much higher percent adsorption of *C. rugosa* lipase was reported in a previous work (*ca*. 80 % onto silica), which was due to mesoporous and fibrous morphology of SBA-15.[16]

Therefore, besides electrostatics, the morphology of the support seems to plays an important role in the adsorption and should also be considered. It could be a determining factor of the efficiency of enzyme adsorption, once the optimum dispersion conditions were provided.

Effect of immobilization on the catalytic properties of the enzyme

Catalytic properties of an enzyme can be modified by immobilization. These changes may be due to conformational alterations within the enzyme. The kinetic behavior of an immobilized enzyme could differ significantly from that of the same enzyme in free solution. Immobilization can also greatly affect the stability of an enzyme. This is primarily due to the physical prevention of large conformational changes within the protein structure.

The catalytic action of the lipase depends on the interfacial activation that occurs when the lipase binds to a lipid interface *via* the opening of the *α*-helical lid that covers the active site. There are several kinetic models for lipase activity, ranging from Michaelis–Menten kinetics and the first order Ping Pong Bi Bi mechanism[34] to more complicated ones taking into consideration the differences between the interfacial and bulk concentration[35] and also the time of solution penetration into support with immobilized lipase.[36] Although Michaelis–Menten mechanism assumes enzyme reaction in the medium where enzyme and substrate must be part of the same phase and does not take into account the activation of the lipase, it is still commonly used as a simplified model for explanations and the determination of the kinetic parameters.

Free lipase and lipase immobilized onto alumina obeyed Michaelis–Menten kinetics. Two parameters, V_{max} and K_m, for the free and immobilized *C. rugosa* lipase were determined and compared. The V_{max}, defined as the highest possible rate of enzyme activity, occurs when the enzyme is saturated with the substrate, and reflects the intrinsic catalytic character of the enzyme. K_m is defined as the substrate concentration that gives a reaction rate of $0.5V_{max}$, implying an affinity between enzyme and substrate. Values of kinetic parameters could not be directly compared with those previously reported for free and immobilized enzyme if the assay conditions (temperature, reaction system used) were not the same, but the trend of the modification of the catalytic property is comparable.

V_{max} and K_m were calculated from the experimental data using the Lineweaver–Burk equation. In this study, the K_m value of the immobilized lipase was similar to that of free lipase, 0.264 *vs*. 0.241 mM, suggesting that enzyme affinity towards the substrate was not altered by its adsorption onto alumina. Similar results were reported for *C. rugosa* lipase immobilized on zirconia, magnetized dacron and niobium oxide,[16,37,38] since adsorption causes little or no conformational change of the enzyme or destruction of its active site.[39] The V_{max} value for the free lipase was 172.0 U mg^{-1} that decreased after immobilization onto

alumina to 28.8 U mg^{-1}, which might be a consequence of increased rigidity of the enzyme. A reduced maximal velocity of immobilized enzymes was reported for *C. rugosa* lipase adsorbed onto silica, zirconia and niobium oxide.[16,38]

Thermal stability

One of the advantages of enzyme immobilization is its improved stability. Thermal stability of the immobilized lipase was evaluated, as shown in Fig. 3. Immobilized lipase was incubated for 2 h at 50 and 60 °C and the lipase activities were measured at 20 min intervals.

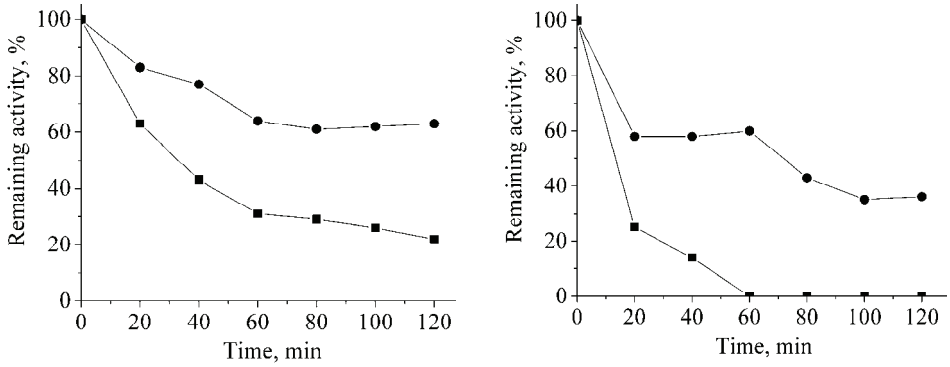

Fig. 3. Thermal stability of free (■) and immobilized lipase (●) at: a) 50 and b) 60 °C.

After 2 h of incubation at 50 °C, the remaining activity of free lipase was 31 %, but the remaining activity of immobilized lipase was 73 % (Fig. 3a). In terms of the half-life at 50 °C, the $t_{1/2}$ of lipase increased after immobilization onto alumina 5.6 times (from 32 min to 180 min).

After 1 h of incubation at 60 °C, the free lipase was not active at all while 60 % of its activity remained when immobilized (Fig. 3b). Even after 2 h, the activity of the lipase immobilized onto alumina was significantly preserved (nearly 40 %). In terms of half-life at 60 °C, the $t_{1/2}$ of the lipase increased after adsorption 5.8 times (from 14 to 81 min).

Thermal stability of lipase on support was significantly improved. Improvement in thermal stability seems to be a result of restricted movement of the protein after adsorption, which prevents conformation changes and unfolding.

Reuse stability

One of the most useful advantages of immobilization of an enzyme is its reusability. Adsorption on a matrix as an immobilization method is usually considered a method with poor reuse potential. However, the remaining activity of the enzyme on alumina was higher than 80 % after seven reuses and almost 50 %

after 11 cycles of use (Fig. 4). As the strength of protein–support interactions can be rated from the ability of the enzyme to resist removal – leaching, this result suggests a significant potential of lipase immobilized on alumina to be reused in biotechnological processes.

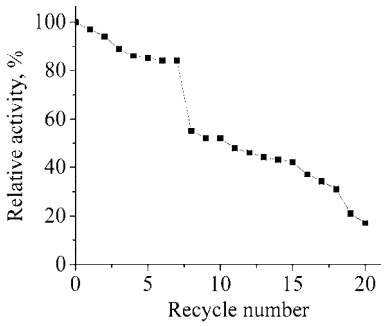

Fig. 4. Effect of reuse on the activity of immobilized lipase.

CONCLUSIONS

In this study, *C. rugosa* lipase was successfully adsorbed onto alumina as a support. The lipase adhered under both repulsive and attractive electrostatic conditions with comparable efficiency. Patchwork surface with mixed positive and negative charges was supposed to be responsible for this behavior. The zeta potential was proven an indicator of protein binding, *i.e.*, a shift in the zeta potential of the support towards that of enzyme was always registered. The morphology of the support also seems to play an important role in the adsorption process. Kinetic constants provided clear evidence that the enzyme affinity was not altered by its adsorption onto alumina, while V_{max} of the lipase decreased 6-fold after immobilization. The thermostability of the adsorbed lipase was improved more than 5 times at 50 and 60 °C. The results suggest a significant potential for reuse of the lipase immobilized onto alumina.

Acknowledgment. This research was financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Projects Nos. III 43004 and III 45012).

ИЗВОД

АДСОРПЦИЈА ЛИПАЗЕ ИЗ *Candida rugosa* НА ГЛИНИЦИ: УТИЦАЈ ПОВРШИНСКОГ НАЕЛЕКТРИСАЊА

ЛИДИЈА Т ИЗРАЕЛ ЖИВКОВИЋ[1], ЉИЉАНА С ЖИВКОВИЋ[2], БОЈАН М. ЈОКИЋ[3], АНДРИЈА Б. САВИЋ[2] и ИВАНКА М. КАРАЦИЋ[1]

[1]*Универзитет у Београду, Медицински факултет, Институт за хемију у медицини, Вишеградска 26, 11000 Београд,* [2]*Универзитет у Београду, Институт за нуклеарне науке Винча, 11000 Београд и* [3]*Универзитет у Београду, Технолошко–металуршки факултет, Карнегијева 4, 11000 Београд*

У овом раду испитиван је утицај површинског наелектрисања глинице на адсорпцију *Candida rugosa* липазе на основу зета потенцијала оба учесника процеса. Липаза се адсорбује у условима и електростатичког привлачења и одбијања, при чему се вредност зета потенцијала носача помера ка вредности истог ензима. Овакво понашање је објаш-

њено хетерогеном расподелом површинског наелектрисања молекула липазе. Посебна пажња је посвећена проучавању утицаја имобилизације на кинетичке параметре липазе. Афинитет ензима није промењен након адсорпције, али је максимална брзина смањена. Термостабилност адсорбоване липазе је побољшана. Потврђена је и могућност вишеструке употребе имобилизоване липазе.

(Примљено 22. фебруара, ревидирано 16. априла, прихваћено 30. априла 2015)

REFERENCES

1. S. Roger, S. Van Pelt, *Chem. Soc. Rev*. **42** (2013) 6223
2. H. Ghamgui, N. Miled, M. Karra-Chaabouni, Y. Gargouri, *Biochem. Eng. J*. **37 (**2007) 34
3. S. Datta, L. R. Christena, Y. R. S. Rajaram, *Biotech* **3** (2013) 1
4. L. L. Hench, *J. Am. Ceram. Soc.* **74** (1991) 1487
5. A. Marti, *Injury* **31** (2000) 33
6. B. Andualem, A. Gessesse, *Biotechnology* **11** (2012) 100
7. H. Fariha, A. S. Aamer, H. Abdul, *Enzyme Microb. Techol*. **39** (2006) 235
8. A. Pandey, S. Benjamin, C. R. Soccol, P. Nigam, N. Krieger, V. Soccol, *Biotechnol. Appl. Biochem*. **29** (1999) 119
9. A. Rajendran, A. Palanisamy, V. Thangavelu, *Braz. Arch. Biol. Technol*. **52** (2009) 207
10. M. Rabe, D. Verdes, S. Seeger, *Adv. Colloid Interface Sci*. **162** (2011) 87
11. S. Hudson, E. Magner, J. Cooney, B. K. Hodnett, *J. Phys. Chem., B* **109** (2005) 19496
12. N. Schultz, G. Metreveli, M. Franzreb, F. H. Frimmel, C. Syldatk, *Colloids Surfaces, B* **66** (2008) 39
13. P. Zeliszewska, A. Bratek-Skicki, Z. Adamczyk, M. Ciesla, *Langmuir* **30** (2014) 11165
14. K. Rezwan, A. R. Studart, J. Voros, L. J. Gauckler, *J. Phys. Chem., B* **109** (2005) 14469
15. M. E. Aubin-Tam, K. Hamad-Schifferli, *Langmuir* **21** (2005) 12080
16. L. Izrael Živković, Lj. Živković, B. Babić, M Kokunešoski, B. Jokić, I. Karadžić, *Biochem. Eng. J*. **93** (2015) 73
17. R. Laucournet, C. Pagnoux, T. Chartier, J. F. Baumard, *J. Am. Ceram. Soc.* **83** (2000) 2661
18. I. Karadžić, A. Masui, L. Izrael Živković, N. Fujiwara, *J. Biosci. Bioeng.* **102** (2006) 309
19. Z. Knežević, N. Milosavić, D. Bezbradica, Z. Jakovljević, R. Prodanović, *Biochem. Eng. J*. **30** (2006) 269
20. M. M. Bradford, *Anal. Biochem*. **72** (1976) 248
21. S. Fazio, J. Guzman, M. T. Colomer, A. Salomoni, R. Moreno, *J. Eur. Ceram. Soc.* **28** (2008) 2171
22. B. Jachimska, M. Wasilewska, Z. Adamczyk, *Langmuir* **24 (**2008) 6866
23. M. Kosmulski, *Chemical Properties of Materials Surfaces*, Marcel Dekker Inc., New York, 2001, pp. 223–265
24. Z. Adamczyk, *Adv. Colloid Interfac*e *Sci*. **100**–**102** (2003) 263
25. Z. Adamczyk, M. Nattich, M. Wasilewska, M. Zaucha, *Adv. Colloid Interface Sci*. **168** (2011) 3
26. J. N. Israelachvili, *Intermolecular and Surface Forces*, Academic Press, London, 1992
27. D. Kovačevic, D. Mazur, T. Preočanin, N. Kallay, *Adsorption* **16** (2010) 405
28. I. Đapić, D. Kovačević, *Croat. Chem. Acta* **84** (2011) 185
29. J. Lutzenkirchen, T. Preočanin, D. Kovačević, V. Tomišić, L. Lovgren, N. Kallay, *Croat. Chem. Acta* **85** (2012) 391
30. R. J. Pugh, *Dispersion and Stability of Ceramic Powders in Liquids,* in *Surface and Colloid Chemistry in Advanced Ceramics Processing,* R. J. Pugh, L. Bergstrom, Eds., Marcel Dekker Inc., New York, 1994, pp. 127–192

31. T. Zheng, *M.Sc. Thesis*, Luleå University of Technology, Luleå, 2008
32. Lj. Čerović, G. Lefèvre, A. Jaubertie, M. Fédoroff, S. Milonjić, *J. Colloid Interface Sci*. **330** (2009) 284
33. B. D. Bowen, N. Epstein, *J. Colloid Interface Sci.* **206** (1998) 314
34. S. Al-Zuhair, K. B. Ramachandran, M. Hasan, *Biochem. Eng. J.* **19** (2004) 81
35. S. Al-Zuhair, K. B. Ramachandran, M. Hasan, *Process Biochem. (Oxford, U.K.)* **38** (2003) 1155
36. A. E. Wiacek, *Powder Technol.* **212** (2011) 332
37. M. C. Pimentel, A. B. Leao, E. H. Melo, W. M. Ledingham, J. L. Lima Filho, M. Sivewright, J. F. Kennedy, *Artif. Cells, Blood Substituents Immobilization Biotechnol*. **35** (2007) 221
38. M. Michele, D. Urioste, L. T. Andrade Souya, A. A. Mendes, H. F. De Castro, *Enzyme Res.* (2011) 216435
39. S. C. Wu, Y. K. Li, *J. Mol. Catal., B* **54** (2008) 103.