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Research Article

Investigation of compatibility of lipase with commercial detergents, surfactants and oxidizing agent as a detergent additive

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ABSTRACT

Herein, it is aimed to perform some commercial liquid and solid laundry detergents, surfactants, oxidizing agent and pH/temperature studies of lipase from porcine pancreas. For this, optimum pH and temperatures of lipase were investigated in the range of pH 5-13 and temperature 30-80°C. The enzyme stability and compatibility were evaluated in the presence of 8 commercial laundry detergents, triton X-100, tween 20, tween 80, tergitol, sodium deoxycolate, sodium dodecyl sulfate and oxidizing agent H_2O_2 for 1 h at 40°C. Optimum pH and temperatures of enzymes was recorded pH 10 and 40°C, respectively. The findings revealed that lipase was generally stable in detergents and increased by more than 20% in liquid detergent 1 and 3 compared to the control. In addition, enzyme in triton X-100, tween 20, tween 80, and tergitol at 5%(v/v) concentration exhibited about 237%, 281%, 207% and 237% relative activities and activity at 5% H202(v/v) was inhibited by only about 13%. Consequently, pancreatic lipase can be a good choice in enzyme immobilization studies and various biotechnological purposes, especially in detergent applications.

Keywords: Lipase, laundry detergent, surfactant, bleaching agent, detergent industry.

Deterjan katkı maddesi olarak lipazın ticari deterjanlar, yüzey aktif maddeler ve oksitleyici ajan ile uyumluluğunun araştırılması

ÖZ

Bu çalışmada domuz pankreasından elde edilen lipazın ticari sıvı ve katı deterjanlar, yüzey aktif maddeler, oksitleyici ajan ve pH/sıcaklık çalışmalarının gerçekleştirilmesi amaçlanmıştır. Bu amaçla enzimin optimum pH ve sıcaklık çalışmaları sırasıyla pH 5-13 ve 30-80°C sıcaklık aralıklarında araştırılmıştır. Enzimin 8 adet ticari çamaşır deterjanı, triton X-100, tween 20, tween 80, tergitol, sodyum deoksikolat, sodyum dodesil sülfat ve oksitleyici ajan H_2O_2 varlığında 1 saat ve 40°C'de stabilite ve uyumluluğu değerlendirildi. Elde edilen bulgulara göre lipaz deterjanlar içerisinde genel olarak stabildir ve kontrole göre sıvı deterjan 1 ve 3 içerisinde aktivitesini %20'den fazla artırmıştır. Aynı zamanda enzim %5 (v/v) konsantrasyonda triton X-100, tween 20, tween 80, and tergitol varlığında sırasıyla yaklaşık %237, %281, %207 and %237 bağıl aktivite göstermiştir ve aktivite %5 (v/v) H_2O_2 konsantrayonunda sadece yaklaşık %13 inhibe olmuştur. Sonuç olarak; pankreatik lipaz, enzim immobilizasyon çalışmaları ve özellikle deterjan uygulamaları başta olmak üzere çeşitli biyoteknolojik amaçlar için iyi bir seçenek olabilir.

Anahtar Kelimeler: Lipaz, çamaşır deterjanı, yüzey aktif maddeler, ağartıcılar, deterjan endüstrisi.

1. INTRODUCTION

Lipases (E.C.3.1.1.3) are triacylglycerol ester hydrolases that interact with carboxyl ester bonds in triacylglycerols to form fatty acids and glycerol. In addition to their hydrolytic activities on triglycerides, lipases are of the industrial biocatalysts and are involved in catalyzing an array of chemical reactions such as esterification, transesterification, peptide synthesis, and site-selective, chemo-selective stereo-selective transformations.[1,2] Due

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to their broad substrate specificity and ability to catalyze multiple reactions both in water soluble/insoluble systems, lipases are widely used for various industrial applications. For instance, the relevant enzymes are used in detergent, food, paper, cosmetics, pharmaceutical, leather, waste and various biotechnological processes at industry.[3,4]

Not only lipases but also enzymes such as protease, amylase, and cellulase are used to enhance the efficiency of the detergents.[5] In particular, the main commercial use of hydrolytic lipases includes detergent processing and accounts for one-third of total lipase sales. The relevant enzymes are used in laundry and dishwashing detergents, in which the enzymes provide significant functions in removal of fatty residues.[6] In this regard, recombinant DNA technology and protein engineering in the production of enzymes for various industrial purposes have been great interests of the researchers. So far, enzymes are produced from a single microbial source using recombinant DNA technology in commercial detergents. Since they enable yielding high product, genetic modification opportunity and large amount of protein as well as they are cost-effective, recombinant DNA techniques are widely preferred.[7-9] Commercial detergents use the lipases produced by recombinant method Lipolase from *Humicola lanuginosa*, Lumafast from *Pseudomonas mendocina* and Lipomax of *Pseudomonas glumae*.[10] On the other hand, protein engineering, improves the functions of the enzyme with some modifications in the molecular structure of the enzyme. In this regard, the studies linked to the provide the desired chemical structure that can be applied to different industrial areas are of the recent interests among researchers.[11] Especially, the enzyme immobilization is of the notable purposes. The catalytic activity, reusability, and resistance to various environmental conditions are increased as a result of the physical or chemical binding and capture of the enzyme to a water-insoluble carrier with enzyme immobilization.[12,13] As previously reported, significant advances in the properties of enzymes have been made by using a wide variety of immobilization techniques, recombinant DNA technique and protein engineering. Consequently, their potential for industrial adaptation has been increased.[14] In enzyme immobilization, enzyme selection affects the choice of immobilization strategy. If the enzyme shows low activity initially and has low tolerance to various chemical agents, then an immobilization method that does not affect the enzyme activity is chosen.[15]

The chosen immobilization technique does not always improve the properties of the enzyme available, sometimes it only ensures its reusability.[15] In the case of an enzyme to be used for the detergent industry, it is initially required to construct an immobilization technique with an enzyme whose compatibility is known in detergents. A good detergent enzyme should generally maintain its stability against high temperature-alkaline environment, metal ions, organic solvents, surfactants, inhibitors and other chemical agents.[5,8,9,14] Therefore, as an initial step of the researches, these characteristics of the enzyme should be well- documented in detergent researches. In this study, the characteristics of the free enzyme in detergent chemicals and its suitability for immobilization studies will be evaluated. Based on this, activity of lipase from porcine pancreas were assessed in the presence of some commercial liquid and solid laundry detergents, surfactants and oxidizing agent. Herewith the current report, the compatibility of pancreatic lipase in detergents and its potential for biotechnological applications were investigated.

2. MATERIALS AND METHODS

2.1. Chemicals

The chemicals used for the experiments were procured from Sigma Chemical Co. (St. Louis, MO, USA). The relevant chemicals were as follows: Lipase from porcine pancreas, bovine serum albumin (BSA), p -nitrophenyl palmitate (p-NPP), p-nitrophenol, triton X-100, tween 20, tween 80, tergitol, sodium deoxycolate (DOC), sodium dodecyl sulfate (SDS), HCl, NaOH, acetate, tris, glycine, ethyl alcohol, and acetonitrile. Bradford reagent was purchased Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, CA, USA). For all experimental stages, double-distilled water (18.2 MΩ; Millipore Co., USA) was used.

2.2. Lipase activity assay

The lipase activity was performed in a spectrophotometrically assay using p-NPP as the substrate. The p-NPP at 10 mM concentration was prepared by mixing ethyl alcohol and 50 mM glycine-NaOH buffer solutions (pH 10) in acetonitrile at a ratio of 1:4:95 (v/v/v). 50 µL of enzyme solution was added to 950 µL of this mixture. The samples were left for incubation at 37°C for 20 min and then, the absorbances regarding lipase activity were recorded at 405 nm. One unit (U ml^{-1}) of alkaline lipase corresponds to the required-amount of enzyme to the release of 1 mol p-nitrophenol per minute under optimum conditions.[16] The protein concentration of enzyme was quantified according to the Bradford method and BSA concentrations (2-12 µg/mL) were used to achieve for standard calibration curve for estimation of protein concentration at 595 nm.[17]

2.3. Determination of optimal pH and temperature for lipase

The lipase activity was assayed for 20 minutes over a range of pH 5-13 at 37°C. Relative activity was measured as described in the lipase activity assays. The buffer solutions (50 mM) of sodium acetate, Tris-HCl, and glycine-NaOH were used for pH 5-6, 7-9, and 10-13, respectively. The effect of temperature on the activity of

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lipase was evaluated at the temperatures ranging from 30 to 80°C for 20 min. ultimately, the relative activity of lipase was quantified at pH 5-13 at 37°C and the activity of the non-heated enzyme was expressed as 100%.[18]

2.4. Stability and compatibility of lipase with commercial detergents

The lipase stability in presence of commercialized liquid and solid detergents were examined. In this regard, some commercial liquid and solid laundry detergents were used. Of the commercial laundry detergents corresponding to the 1-4 liquid and 5-8 solid detergents, respectively. Firstly, the detergents were diluted to a final-concentrations (7 mg/mL; solid detergents; 10% (v/v); liquid detergents). Then the lipase-added detergents were left for incubation at 40°C for 1 h. Ultimately, the relative activity of lipase was quantified under optimum conditions. The activity of lipase without detergent addition was expressed as 100%.

2.5. Effect of some surfactants and oxidizing agent hydrogen peroxide on lipase activity

The effect of some non-ionic surfactants (5% (v/v) triton X-100, tween 20, tween 80, and tergitol) and ionic surfactants (5% (w/v) sodium dodecyl sulfate (SDS) and 5% (v/v) sodium deoxycolate (DOC) and also the effect of oxidizing agent (5% (v/v) H_2O_2) on lipase activity were assessed after incubation at 40°C for 1 h. Finally, the relative activities of lipase were determined under optimum conditions. The activity of lipase without and any addition was expressed as 100%.

2.6. Statistical analysis

The data were means of three replicates corresponding to the two technical replicates (duplicate measurement per each replicate). The means of the experimental groups were compared using one-way variance analysis (SPSS 16.0).

3. RESULTS AND DISCUSSION

3.1. Determination of optimum pH and temperature of lipase

To determine the optimal catalytic conditions of lipase, the effect of pH and temperature on activities of lipase were evaluated (Figure 1A-B). A range of 5 to 13 of pH was used to reveal the effect of pH on lipase. As shown in the Figure 1A; the optimal pH value of lipase was found to be 10. The enzyme retained more than about 80% of its activity in the pH range of 7-12. Especially, it retained about 95% of its activity, especially at pH 9 and 11. Also, it exhibited 67% and 71% activities in the acidic medium pH 5 and 6, respectively, whilst it retained about 59% of its activity in the highly alkaline medium pH 13.

The optimum temperature of lipase was found to be 40 °C (Figure 1B). The enzyme exhibited very high activity at the temperature range of 30-60°C. It exhibited 93% activity at 50 and 60°C. At higher temperatures, it retained more than 60% of its activity. After the optimum conditions were determined, the activity and specific activities of the lipase were determined as 13.64 U/mL and 10.91 U/mg protein, respectively. However, in the previous reports, lipases have been produced from different sources with a wide range of different pH/temperature activity and stability.[19,20]

The potential uses of the enzymes to be applied for industrial processes are likely dependent on the adaptation of the enzymes to the difficult and harsh reaction conditions.[4,6] For this reason, enzymes are subjected to biotechnological processes such as enzyme immobilization, recombinant DNA technology and nanotechnology in order to increase the adaptation and tolerance of enzymes to industrial applications.[7,11] It can be emphasized once again that lipase might be a good source for biotechnological purposes with the high temperature and acid/alkaline environment stability exhibited by the enzyme.

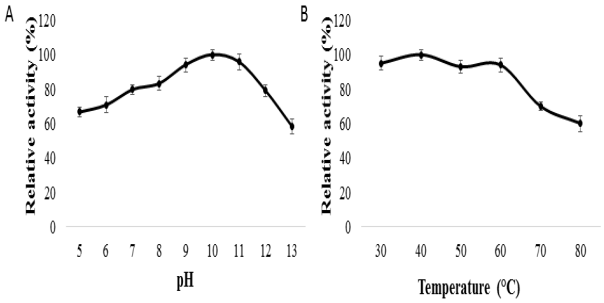

Figure 1. Effects of pH (A) and temperature (B) on the activities of lipase. The activities of the non-heated lipase were taken as 100%.

3.2. Detergent stability and compatibility

To examine suitability and compatibility of the lipase in detergents, lipase was pre-incubated for 1 hours at 40°C with various solid and liquid commercial laundry detergents. The findings of the current revealed that lipase was generally stable (Figure 2). The lipase activity increased significantly in liquid detergent 1, 3 and 4, in comparison to the control ($p<0.001$), while it retained 75% of its initial activity in detergent 2. The enzyme showed more than 120% activity in detergents 1 and 3. The activity in solid detergent 5, 6, 7 and 8 was about 64%, 88%, 89% and 68% respectively.

While biological detergents use enzymes that break down stains, non-biological detergents deal with tough stains with higher temperatures or longer wash times. Therefore, biological detergents are less harmful to the environment and are more economical. In recent years, lipases have been preferred in industrial applications due

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to their biodegradable, non-toxic, large amount of production and substrate diversity.[21] They are used in detergents to remove oil-based dirt and work by forming a fabric-lipase complex that creates a barrier on clothing. An ideal detergent enzyme is expected to be able to adapt to high washing temperatures and alkaline environment conditions. [5,14] A wide variety of alkaline and thermotolerant lipases have been studied in previous researches and tested in different industrial areas. The lipase from *Geobacillus stearothermophilus* FMR12 had optimum activity at 70 °C and pH 9 and it was stable until 90 °C and pH 11 and in commercial detergents.[9] The lipase from *Aeromonas caviae* LipT51 lipase activity was increased 2.5 times in the presence of laundry detergent and it retained all of initial activity in dish washing and handwashing detergent.[22] The purified lipase from *Bacillus licheniformis* strain NCU CS-5 retained more than 50% of its activity in the presence of commercial detergents at 5 or 25 °C after 3 h incubation.[23] According to findings of the current study, lipase from porcine pancreas is suitable for detergent applications thanks to its high activity and stability in commercial solid and liquid washing detergents. In order to improve the effectiveness of lipase-containing detergents and to understand the deactivation mechanism of lipase under washing conditions, it is necessary to investigate the important kinetic and thermal, hydrodynamic and detergent/surfactant characteristics of the lipase at the oil-water interface.[24]

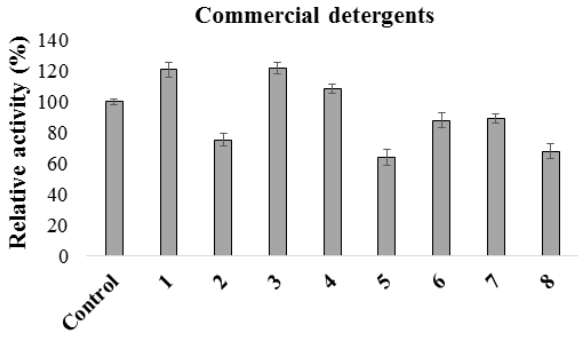

Figure 2. The effect of commercialized liquid and solid detergents on the enzymatic activity. The detergents were used final-concentrations 7 mg/mL for solid detergents and 10% (v/v) for liquid detergents. The activity of lipase without detergent addition was expressed as 100%.

3.3. Effect of some surfactants and oxidizing agent hydrogen peroxide on lipase activity

For determination of the compatibility of enzymes with detergents, their competitiveness and tolerance with detergent chemicals like surfactants, blenching and oxidizing agents are investigated. These chemicals can destroy lipase activity by altering the tertiary structure of lipase. To evaluate the suitability of the lipase in detergent, lipase was tested in presence of some non-ionic surfactants, ionic surfactants and oxidizing agent H_2O_2 by incubating at 40°C for 1 h. As in Figure 3, the enzyme activity increased more than 2-folds in the presence of non-ionic surfactants compared to the control ($p<0.001$). The lipase in triton X-100, tween 20, tween 80, and tergitol at 5% (v/v) concentration displayed about 237%, 281%, 207% and 237% relative activities, respectively. Various lipases produced from different sources exhibited varying characteristics in surfactants. Some reports are available to prove the positive effect of surfactants on the lipase. The lipase from *Haloferax mediterranei* CNCMM 50101 in 10 % (v/v) triton X-100, tween 20, 60 and 80 retained their initial activities 40 °C for 1 h.[20] In another study, lipase isolated from *Bacillus* strain showed about 84%, 103%, 98% and 1% relative activities in 1% (v/v) Triton X-100, Tween 20 and 80, and SDS, respectively.[25] Lutensol XP80 and Triton X-100 strongly was activated the lipase from *Pseudomonas aeruginosa* for 1 h (up to 40 and 30% against the control, respectively).[26] In contrast, lipase from *Staphylococcus* sp. strain CJ3 was strongly inhibited by Triton X-100.[27] In a previous study, it was reported that hydrogen bonds easily form between non-ionic surfactants and protein molecules, increasing conformational flexibility in the active site.[28] In this study, lipase lost about 32% and 25% of its initial activities in the presence of the ionic surfactants DOC and SDS, respectively. Lipase from *Geobacillus stearothermophilus* FMR12 exhibited about 94% and 93% relative activity in the presence of 5% DOC and SDS, while it had %92 and %79 activity at 10% surfactant concentration, respectively.[9] According to the mechanism proposed by Bajpai and Tyagi [29], the negative charges of SDS react with the positive charges of some metal ions such as calcium and magnesium in the washing water and deactivate them. It has been suggested that these ionic surfactants denature the enzyme and reduce the activity by interacting with the forces that preserve the three-dimensional structure of the enzyme.[30] The other mechanism is that ionic interactions between ionic surfactants and enzyme play a role in the inactivation of globular proteins.[31]

Another parameter for detergent applications is the tolerance of enzymes against oxidizing agents. The lipases were generally inhibited in the presence of oxidizing agent H_2O_2 and other bleaching agents at high concentration.[14,32] For instance, while the lipase from *Geobacillus stearothermophilus* showed 65% activity in 5% H_2O_2 (v/v), it lost more than 75% of its activity in the presence of 10% H_2O_2 (v/v).[9] In this study, it was noted that lipase activity at 5% H_2O_2 (v/v) was inhibited by only about 13% after 1 h at 40°C. According to the current findings, it is clear that the tolerance level of the lipase from porcine pancreas against ionic and non-ionic surfactants and oxidizing agent H_2O_2, which is in a

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concentration equivalent to the washing conditions, is high.

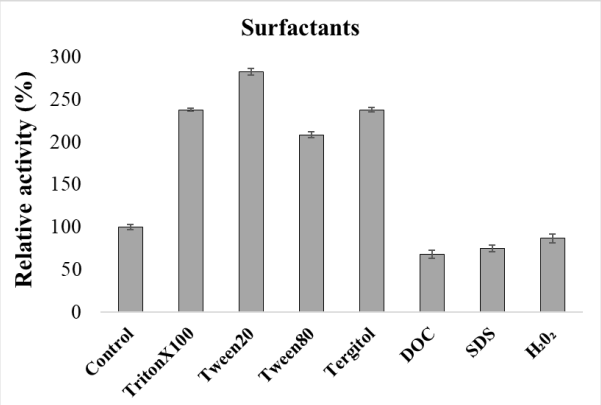

Figure 3. The effect of non-ionic and ionic surfactants on the enzymatic activity. The activity of lipase without any addition was expressed as 100%.

4. Conclusion

As the demand for enzymes with improved characteristics that are resistant to difficult reaction environments in the detergent industry increases, biotechnological researches on detergent enzymes are increasing. In particular, the researchers focus on the properties of enzyme such as kinetics, pH/temperature, and behavior against various chemical agents in washing water. Herewith the findings, it was concluded that lipase exhibited significant stability and compatibility in the presence of various detergents, surfactants and oxidizing agents H_2O_2. Especially for the detergent industry, pancreatic lipase can be a good choice in enzyme immobilization studies and various biotechnological purposes.

Conflict of interest

I declare that there is no a conflict of interest with any person, institute, company, etc.

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