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Application of Natural Kaolin as Support for the Immobilization of Catalase from Bovine Liver

ALI SAVRAN, SALIH ALKAN, HALIT DEMIR* and HASAN CEYLAN
Department of Chemistry, Science and Arts Faculty
Yüzüncü Yil University, Van, Turkey
Fax: (90)(432)2251114; Tel: (90)(432)2251026/2260
E-mail: halitdemir@hotmail.com

Catalase from bovine liver was immobilized on to natural kaolin by physical adsorption method. About 80% of the protein content was immobilized on to support. The activities of immobilized catalase were determined in the reaction mixture containing substrate hydrogen peroxide and free catalase. The effects of reaction temperature, thermostability, stability in organic solvent, leaching and storage studies of immobilized catalase were investigated. Kaolin-immobilized catalase exhibited activities higher by four folds than free catalase after thermal stability test at 70°C. Immobilized catalase was found to be stable in hexane at room temperature up to 12 d and also showed higher stability than free catalase in the storage study. Leaching studies showed that the immobilized catalase remained fully active even after being washed by 20 mL of solvent. The experimental results showed that physical adsorption is suitable for the attachment of enzyme on to kaolin.

Key Words: Clay, Kaolin, Immobilized enzyme, Catalase.

INTRODUCTION

Enzymes are often immobilized on to or into solid supports. The supports used for immobilizing enzyme should process mechanical strength, microbial resistance, thermostability, chemical durability, chemical functionality, low cost, hydrophylicity, regenerability and a high capacity of enzyme[1]. Many methods are available for enzyme immobilization. The various methods devised for enzyme immobilization may be subdivided into two general classes: chemical methods, where covalent bonds are formed with the enzyme, and physical methods, where weak interactions between support and enzyme exist including hydrogen bonding, van der Waals' forces and hydrophobic interactions[2]. Immobilization of enzyme through physical methods is still most commonly used because it is the easiest to perform and the least expensive. Both organic and inorganic materials such as porous glass, cellulose, silica gels and hydro gels are used for preparation of immobilized enzymes[3, 4].

Immobilized catalase has useful applications in various industrial fields in the removal of hydrogen peroxide used for oxidizing and bleaching and in the

analytical field as a component of hydrogen peroxide or glucose biosensor[5,6]. Catalase has been immobilized on numerous materials such as magnesium silicate[7], chitosan film[8], hydroxyapatite[9], alumina, gelatin, polyacrylamide, egg shell[10] and perlite[11].

Natural kaolin is inexpensive and shows good potential as support material. At present, strong interest in such natural supports is due to demand in many industrial applications. In this study, catalase from bovine liver was immobilized on kaolin and was physico-chemically characterized.

EXPERIMENTAL

Catalase from bovine liver (E.C.1.11.1.6) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The natural kaolin was bought from Eczacibaşi Co. (İstanbul, Turkey). All other chemicals and solvents used in this study were of analytical grade.

Immobilization of catalase: Catalase from bovine liver (40 mg) was dispersed into 50 mM phosphate buffer (20 mL). The immobilization of catalase was carried out by continuously shaking kaolin (2.00 g) with catalase solution (20 mL) at 100 rpm for 1 h at room temperature. The immobilized catalase was then separated by filtration and washed with 50 mM phosphate buffer at pH 7 to remove the unabsorbed soluble enzyme. The immobilized catalase was then dried in freeze drier.

Protein assay: The amount of protein content before and after immobilization was determined by using the method of Coomassie brilliant blue assay procedure using bovine serum albumin as standard[12]. Defined calculation of protein immobilized in percentage is as follows:

$$\% \text{ Immobilization} = \frac{\text{total amount of protein in supernatant before immobilization} - \text{total amount of protein in supernatant after immobilization}}{\text{total amount of protein in supernatant before immobilization}} \times 100$$

Characterization of the immobilized catalase

Effect of the temperature on activity of catalase: The mixtures were reacted at different temperatures (30, 40, 50, 60 and 70°C) for 5 h at 150 rpm in a water bath shaker. The relative activities were determined as percentage yield at different temperatures compared to the activity of reaction at 30°C as:

$$\text{Relative yield (\%)} = \frac{\text{\% yield at different temperatures}}{\text{maximum \% yield (30°C)}} \times 100$$

Thermal Stability: Catalase was incubated at 30, 40, 50, 60 and 70°C in sealed vials for 1 h. The enzymes were left to cool before activity was determined. The relative activities are determined as percentage yield of activities at different treated temperatures compared to the activity treated at 30°C as:

$$\text{Relative yield (\%)} = \frac{\text{\% yield at different temperatures}}{\text{maximum \% yield (30°C)}} \times 100$$

Stability in Organic Solvent: The catalase preparation was incubated in hexane without shaking for 1–10 days at room temperature. After the incubation, the enzymatic activity was determined at room temperature. The relative activities were determined as percentage yield of activities for 2–10 days compared to the activity at day 1 as:

$$\text{Relative yield (\%)} = \frac{\text{\% yield at number of days}}{\text{maximum \% yield (day 1)}} \times 100$$

Effect of washing on activity of catalase: The kaolin immobilized catalase (20 mg) was washed consecutively with 4.0, 8.0, 12.0, 16.0 and 20.0 mL of hexane. The relative activities were determined and compared to the activity of unwashed immobilized catalase as:

$$\text{Relative yield (\%)} = \frac{\text{\% yield at different mL of hexane}}{\text{maximum \% yield (0 mL)}} \times 100$$

Effect of storage temperature on activity of catalase: This study was conducted to investigate the stability of catalase compared to immobilized catalase at different storage temperatures, –20°C, 0°C and 4°C. The catalases were kept for 60 days. The relative activities are determined and compared to the activity at day 1 for each temperature as:

$$\text{Relative yield (\%)} = \frac{\text{\% yield at different storage temperatures}}{\text{maximum \% yield (storage temperature)}} \times 100$$

RESULTS AND DISCUSSION

Using the Bradford method, the protein content in free catalase before immobilization was determined as 1.35 mg. After immobilization, the protein content in supernatant was found to be 0.02 mg. From the calculation, it was estimated that 98.50% of protein in supernatant from catalase has been immobilized on to the support. The catalase molecules may generally be immobilized on the surface and within the support as the protein molecules of catalase replacing the molecules of water. This natural kaolin was found to exhibit properties which are accommodating for adsorption of about 98.50% protein and may also improve catalytic activity. Kaolin provides good distribution of catalase on mass transfer and prevents catalase particles from aggregation thus helping in the dispersion of catalase in the reaction media. In comparison, free catalase was not easily dispersed as it tends to aggregate, thus causing a decrease in its activity. Enhancement of catalase activity was investigated through immobilization on to natural kaolin support.

The heat energy from the reaction temperature may affect enzymatic rate and functional group of substrate involved in the reaction and, therefore, reactions must be experimented to find the optimum temperature in order to obtain the best yield. The effect of reaction temperature using free catalase and kaolin-immobilized catalase on the activity is shown in Fig. 1. The results show that the highest percentage of activity of both catalases was at 30°C , which is the optimal reaction temperature. The relative activity was found to decrease at temperatures

from 30 to 70°C. At temperatures above 50°C, the relative activity drastically decreased and further decreased with an increase of temperature up to 70°C. Kaolin-immobilized catalase was active even at 70°C, giving 40% of its catalytic activity. It seems that kaolin might be protecting the enzyme against denaturation at higher temperatures.

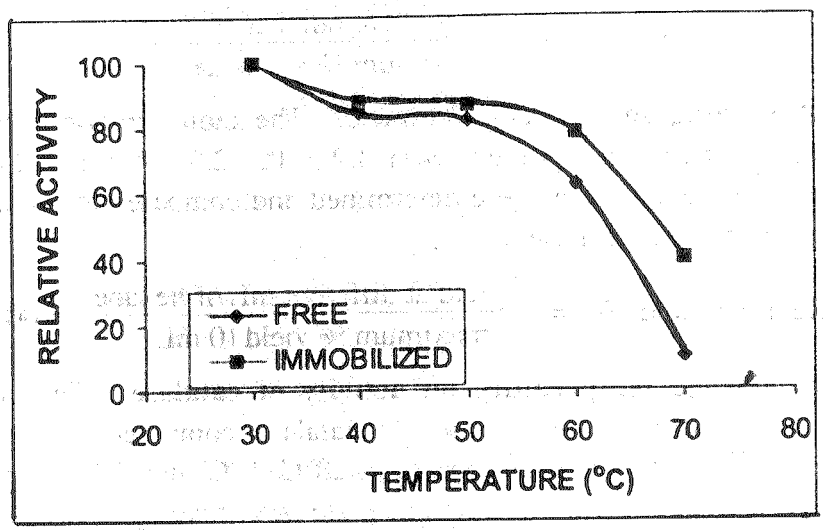

Fig. 1. Effect of temperature on free and immobilized catalase

The kaolin-immobilized catalase showed better thermal stability thae free catalase, which easily undergoes structural changes and aggregation. Fig. 2 shows the thermal stability of kaolin-immobilized catalase compared to free catalase after 1 h incubation at temperatures ranging from 30 to 70°C. However, the thermal stability, which decreases with an increase of temperature from 40 to 70°C, may be due to the disturbance of globular structure of the protein by heat capacity, which causes protein unfolding and leads to loss of enzymatic activity. Immobilized catalase was found to retain its enzymatic activity at high tempera-

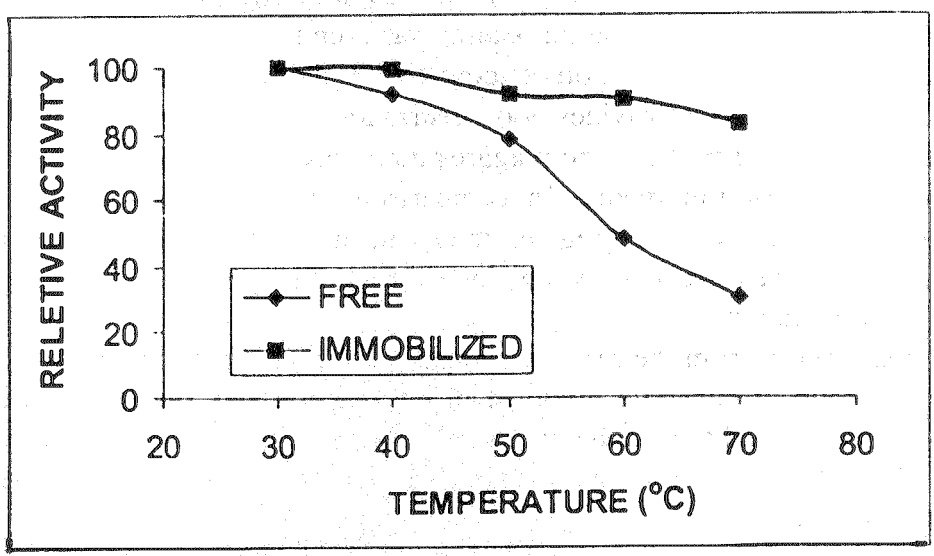

Fig. 2. Per cent relative activity of free and kaolin-immobilized catalase after 1 h incubation at various temperatures

ture compared to free catalase by fourfold higher. At higher temperature, free catalase had probably been denatured while immobilized catalase may be more rigid in terms of conformation and, therefore, was able to remain stable. Although heat could reduce conformational flexibility of free and immobilized catalase, the immobilized catalase was still able to perform its catalytic activity efficiently. Thus, thermal stability can be achieved by immobilization using kaolin and afford the financial cost advantage related to thermostability.

The stability of immobilized catalase incubated in hexane without shaking for 1–10 days at room temperature is shown in Fig. 3. Gradual decrease in activity was determined for kaolin-immobilized catalase; meanwhile, a drastic decrease

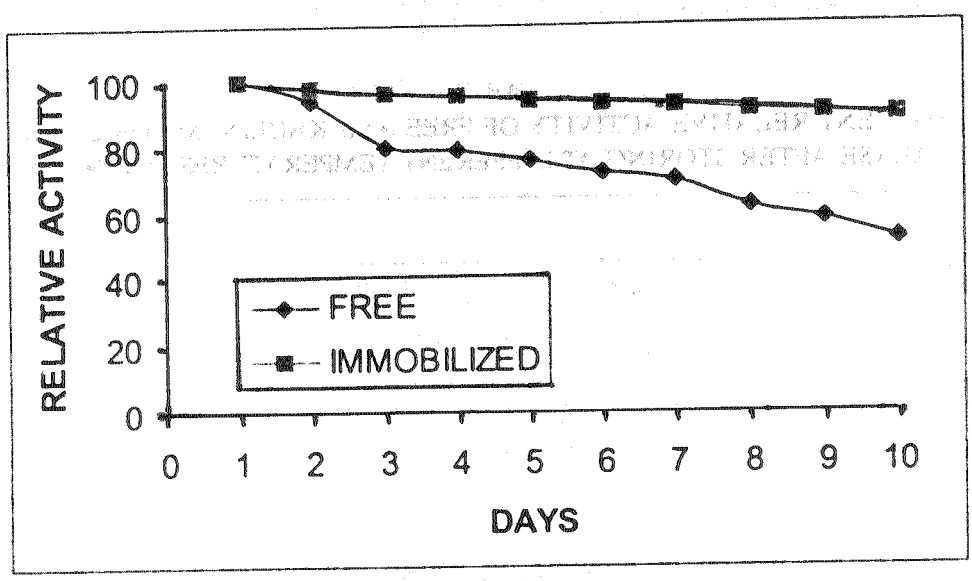

Fig. 3. Per cent relative activity of free and kaolin-immobilized catalase as effected by incubation in hexane at room temperature (25°C)

was exhibited for free catalase starting at day 3. The decrease in activity of catalase was due to the ability of solvent in stripping the essential monolayer of water surrounding enzyme molecules. The supports may trap and prevent the disruption of the enzyme-bound water essential to maintain the three-dimensional structure of enzyme for catalysis as the polar solvents tend to strip water from enzyme molecule. Therefore, the enzyme may be stable in organic solvents than they are in water, and this is the reason why they are used as reaction medium in enzymatic activity.

The effect of washing on activity of free catalase and kaolin-immobilized catalase was investigated using 50 mM phosphate buffer as reaction medium. The catalase preparation has retained full activity of 100 % even after being washed by 20 mL of buffer (5 cycles) indicating the protein of catalase from bovine liver remained adsorbed onto kaolin. This may be due to the hydrophobic interaction between catalase and kaolin that has increased their rigidity, thus avoiding the distortion of catalase protein in buffer. Interestingly, these indicate a good property of kaolin as support, and furthermore, physical adsorption method used in this study is suitable for immobilization of catalase on kaolin.

The ability to be stored for a period of time at a certain temperature is one of the key factors to be considered when using immobilized catalase. Table-1 summarizes the enzymatic activities of catalase after storing for 60 days under various storage conditions (–20°, 0° and 4°C). Both catalases obtained their full activity (100%) at –20°C as commonly practised in the laboratory. Generally, enzymes are still active when kept at low temperature probably because catalase tends to lock to its original conformation, which is catalytically active. Kaolin-immobilized catalase retained 100% catalytic activity when stored at 0°C but free catalase decreased to 95% of its catalytic activity over a period of 60 days. However, the stability of immobilized catalase was still high (80%) at 4°C compared to free catalase.

TABLE-1
PER CENT RELATIVE ACTIVITY OF FREE AND KAOLIN-IMMOBILIZED CATALASE AFTER STORING AT DIFFERENT TEMPERATURES FOR 60 DAYS

Activity			
Catalase	4°C	0°C	–20°C
Free	80	95	100
Immobilized	90	100	100

The effectiveness of an immobilization process depends on the support used. Such increase in stability and activity performed by kaolin-immobilized catalase is favourable, and it exhibits good tolerance to conditions as required in industrial applications. Adsorption of enzyme on kaolin can reduce a large amount of enzymatic activity. Natural kaolin shows a promising future of applying natural resources as support for biocatalyst for various areas as it allows easy immobilization of catalase from bovine liver through a simple and inexpensive method.

REFERENCES

1. A. Kilara, *Process*, 25 (1981).
2. R.G. Burns, Interaction of Soil Minerals with Natural Organics and Microbes, Soil Science Society of America, Madison, WI, p. 439 (1986).
3. J.E. Baileey and D.F. Ollis, Applied Enzyme Catalysis, McGraw-Hill International, Singapore, p. 180 (1986).
4. J.F. Kennedy and E.H.M. Melo, *Chem. Eng. Prog.*, 81 (1990).
5. N. Ertaş, S. Timur, E. Akyilmaz and E. Dinçkaya, *Turkish J. Chem.*, **24**, 95 (2000).
6. T. Santoni, D. Santianni, A. Manzoni, S. Zanardi and M. Mascini, *Talanta*, **44**, 1573 (1997).
7. S.S. Tukel and O. Alptekin, *Process Biochem.*, **39**, 2149 (2004).
8. Ş.A. Çetinus and H.N. Öztop, *Enzyme Microbial Tech.*, **26**, 497 (2000).
9. A. Barroug, E. Lernoux, S. Lemaitre and P.G. Rouxhet, *J. Colloid Interface Sci.*, **208**, 147 (1997).
10. U. Chatterjee, A. Kumar and G.G. Sanwal, *J. Ferment. Bioeng.*, **70**, 429 (1990).
11. L. Tarhan and A. Telefoncu, *Process Biochem.*, **27**, 11 (1992).
12. M.M. Bradford, *Anal. Biochem.*, **72**, 248 (1976).

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