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Adsorption of Catalase Enzyme on Silica Gel

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Immobilization of catalase enzyme on fibrous silica gel was investigated in present study. Simple adsorption has no effect on enzyme activity and appeared to as a new method of immobilizing the enzyme at high ionic strength. The storage stability and the kinetic parameters of immobilized enzyme and its activity were evaluated under varied pH, ionic strength and temperature. These studies indicate that silica gel is a valuable support for simple adsorption of enzymes.

Key Words: Silica gel, Catalase, Clay-supported enzymes, Enzyme immobilization.

INTRODUCTION

Recently, immobilized enzyme systems have been drawn a considerable interest. There are several reasons for the preparation and use of immobilized enzymes. In addition to a more convenient handling of enzyme preparations, the two main benefits are easy separation of the enzyme from the product and reuse of the enzyme[1].

Catalase (hydrogen peroxide oxidoreductase; EC.1.11.1.6) is an important enzyme in biological systems, where it catalyses the reaction shown below:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Immobilized catalase find a number of such as applications in food industries for removal of hydrogen peroxide from food products after cold pasteurization and in the analytical field as a component of hydrogen peroxide or glucose biosensor systems[2]. Catalase has been immobilized on numerous carrier materials such as controlled pore glass[3], asymmetric cellulose membrane[4], nylon membrane[5], alumina[6], ultrafine silica particles[7], polyacrylamide gels entrapment[8]. Inorganic materials have been successfully used for immobilization of enzymes[9]. Inorganic carriers often show good mechanical properties, thermal stability and resistance against microbial attack and organic solvents. Silica gel is an amorphous inorganic polymer composed of siloxane groups (Si–O–Si) in the inward region and silanol groups (Si–OH) distributed on the surface. Chemical modifications

that can occur with this polymer are related to the presence of the disposed silanol groups in its surface.

In this study, catalase was immobilized on the silica gel. The optimal conditions of each step in the immobilization procedure were investigated.

EXPERIMENTAL

Catalase (specific activity, 1880 U/mg) was adsorbed on the silica gel clay (O_2Si amorphous, Merck). In order to determine the optimum conditions for supporting the enzyme, the time course of the retention of catalase by wet micronized silica gel under different conditions (*i.e.* pH, ionic strength, temperature, enzyme-clay weight ratio) are studied. Adsorption is the most economic and simple process and widely employed for immobilization purposes among various methods available for immobilization of enzymes onto solid carriers. There are four procedures that have been used for immobilization of enzymes by adsorption, namely static process, dynamic batch process, reactor loading process and electro deposition process. This treatment was repeated several times until a constant pH was obtained. The silica gel suspensions were prepared at 25 mg/mL and they exhibited a considerable stability. In this study for standart purposes, solutions of catalase enzyme (5 mg/mL) in 0.05 M sodium phosphate buffer, pH 7.0 were added to 25 mg/mL suspensions of micronized silica gel, in a 1:4 volume ratio (2.10^{-5} mg catalase enzyme/ 1.10^{-4} mg silica gel). After shaking, the suspension was centrifuged at 3000 xg at room temperature for 1 h and the supernatant was analyzed for remaining catalase by a spectrophotometric method[10] as described below. This reaction mixture was incubated for 15 min at 25ºC in order to obtain a stable value of the absorbance at 240 nm. The effect of ionic strength on the preparation of the supported catalase was also investigated. As control, the catalase-silica gel complexes were prepared in 0.05 M sodium phosphate buffer, pH 7. The influence of pH on both the amounts of adsorbed catalase and their relative activity has been investigated by performing experiments within 4-9 range of pH. The effect of temperature on the activity of free and immobilized was studied over 20-60ºC. Each activity value was obtained for 15 min incubation at the selected temperature. The amount of enzyme activity to the clay was calculated as the difference between the values obtained in the original preparation and the supernatants.

RESULTS AND DISCUSSION

Effect of pH: By using 0.05 M sodium phosphate buffers effect of pH on to enzyme activity was determined for this purpose. The catalase substrate were used pH value between 4 and 9. The pH activity dependence for catalase enzyme adsorbed on silica gel for and natural enzyme on the

same experimental conditions are shown in Fig. 1. The optimum pH for natural enzyme observed around 7.0. It was seen from figure the optimum pH value for adsorbed enzyme also near by to value of 6.8. Furthermore the pH activity change for adsorbed enzyme is less susceptible is comparable with natural enzyme. Such difference behaviour of natural and adsorbed enzyme may be caused by difference of H^+ and OH^- ions on surface of clay. The decrease in the relative activity of the enzyme with decreasing pH indicates a progressive change in the conformation of the adsorbed enzyme. When the pH falls below the positive charge on the enzyme increases and the attractive columbic interaction with the electronegative surface causes an unfolding of the protein.

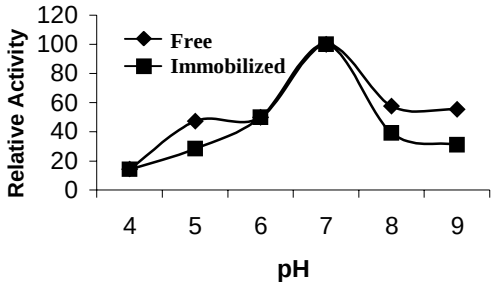

Fig. 1. Effect of pH on free and immobilized catalase

Effect of temperature: The temperature dependence of the activities of the free and immobilized catalase were studied in 0.05 M phosphate buffer (pH 7.0) in the temperature range 20-60ºC and temperature graphs of free and immobilized catalase are showed. Optimum temperature was found at about 30ºC for free and immobilized catalase above 35ºC. It was observed that the loss of the activity of immobilized catalase was lower than that of the free catalase for high temperatures (Fig. 2). The immobilize catalase has a protecting effect at the temperatures at which enzyme deactivation takes place[11].

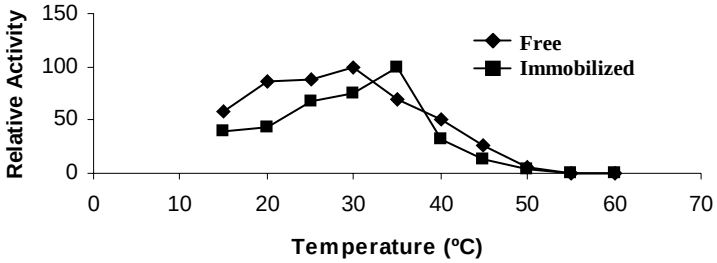

Fig. 2. Effect of temperature on free and immobilized catalase

Effect of ionic strength: The results obtained for both free and immobilized catalase are given in Fig. 3. The silica gel-supported catalase appears more active at high ionic strengths than the free enzyme. Hence,

the adsorption of catalase gives additional stabilization to the activity site against electrostatic interactions. In order to determine the influence of ionic strength on bounded and free enzyme by adding 0.10, 0.15, 0.20, 0.25, 0.30 M sodium phosphate buffers to the reaction medium in constant pH value. According to result we have determined that both free enzymes have shown similar properties to the ionic strength.

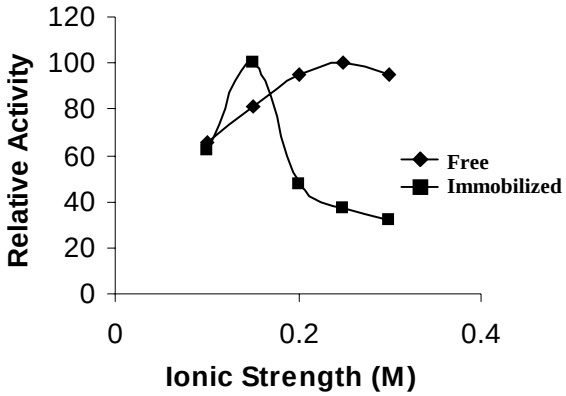

Fig. 3. Ionic strength on free and immobilized catalase

Kinetic parameters: Kinetic constants measured with immobilized enzymes are not true kinetic constants equivalent to those obtained in homogeneous reactions, but are apparent values because of the effect of diffusion and other physical factors. Hence, maximum rate and Michaelis constant should be referred to as apparent V_{max} and apparent K_m. These kinetic constants are related to the effect of substrate concentration on the activity of enzyme when the concentration of enzyme was kept constant. Apparent Michaelis-Menten constant and maximum rate values for free and supported catalase were calculated from the slope of linearized Michaelis-Menten equation, according to the method of Wilxinson modified by Cornish-Bowden.The Lineweaver-Burk plots of the experimental values are shown in Fig. 4 in comparison to the theoretical straight line calculated from the above regression analysis. According to these results, the K_m values for both free catalase and supported catalase are coincident; this suggests both the absence of micro environmental effects on the adsorbed enzyme and an unaltered mechanism of catalysis. This observation also indicates that there is no local increase of catalase concentration which would result in a decrease of the apparent of K_m value. It has been reported that silica gel does not adsorbed catalase in a measurable extent, in spite of the known capacity of catalase to intercalate into other clays altering the layer spacing. In carrier binding method, the electrostatic interaction between the carrier and substrate is considered to be one of the reasons for changes in K_m value upon immobilization. The V_{max} value of

the immobilized enzyme is less than that of the native enzyme V_{max} value, which is in agreement with the activity of this immobilized system. Michailes-Menten equation is written and correlated to determine for K_m and V_{max}.

$$1/V = K_m/V_{max} \cdot 1/S + 1/V_{max}$$

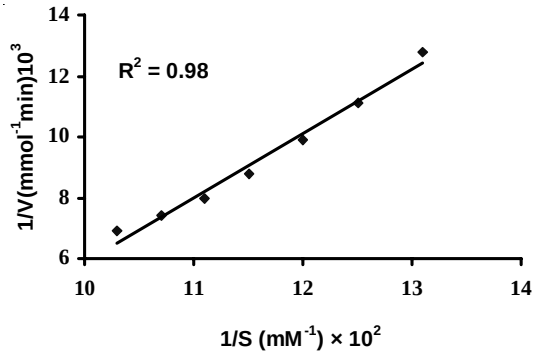

Fig. 4. Lineweaver-Burk plots for free catalase

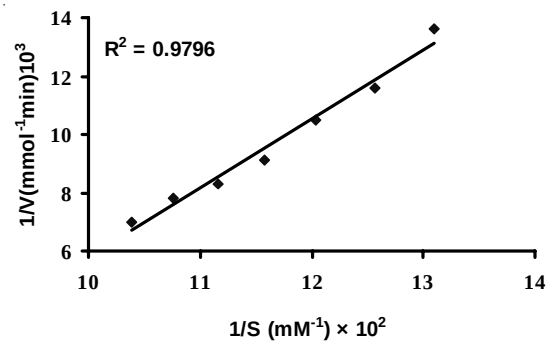

Fig. 5. Lineweaver-Burk plots for immobilized catalase

The value of K_m was found to be 19.23 mM whereas the V_{max} was calculated 20,000 µmol (min mg protein)$^{-1}$ for free catalase. The K_m value was found to be 19.01 mM and the V_{max} value was found to be 32,180 µmol (min mg protein)$^{-1}$ for immobilized catalase in silica gel. As expected, the K_m and V_{max} values were significantly affected after immobilization into silica gel. The change in the affinity of the enzyme to its substrate is probably caused by structural changes in the enzyme introduced by the immobilization procedure or by lower accessibility of the substrate to the active site of the immobilized enzyme[12]. So that the V_{max} value of immobilized catalase very lower than that of free catalase. The K_m and V_{max} values of immobilized catalase are higher than that of free catalase. An increase in K_m indicates that the immobilized enzyme has an apparent lower affinity for its substrate than that of the free enzyme. It may be caused by the steric

hindrance of the active site by the support, the loss of enzyme flexibility necessary for substrate binding or diffusional resistance to solute transport near the particles of the support.

REFERENCES

1. S.W. Park, Y.I. Kim, K.H. Chung, S.I. Hong and S.W. Kim, *React. Funct. Polym.*, **51**, 79 (2002).
2. M.S. Alvesda, M.H. Gill, A.P. Piedace, J.S. Redinha, A.M.O. Brett and J.M. Costa, *J. Polym. Chem.*, **29**, 269 (1991).
3. G. Urbab, H. Kamper, A. Jachimowic and F. Kohl, *Biosens. Bioelectron.*, **6**, 275 (1991).
4. L. Campanella, R. Roversi, M.P. Sammartino and M. Tomassetti, *J. Pharm. Biomed. Anal.*, **18**, 105 (1998).
5. C. Dajun, W. Xiaqin and Z. Zuxin, *Sens. Actuators B*, **13**, 554 (1993).
6. B. Jiang and Z. Zhang, *Eur. Polym. J.*, **29**, 1251 (1993).
7. S.A. Costa, T. Tzanov, A. Paar, M. Gudelj, G.M. Gubitsz and A.C. Paulo, *Enzyme Microb. Tech.*, **28**, 815 (2001).
8. A. Kondo, F. Murakami, M. Kawagoe and K. Higashitani, *Appl. Microbiol. Biot.*, **33**, 63 (1994).
9. A.L. Plant, L.L. Brown, W. Haller and R.A Durst, *Appl. Biochem. Biotechnol.*, **30**, 83 (1991).
10. F.D. Snell and C.T. Snell, Colorimetric Methods of Analysis, Vol. 3, edn. 3 (1971).
11. J.M. Gonzales-Saiz and C. Pizarro, *Eur. Polym. J.*, **37**, 435 (2001).
12. S. Staunton and H. Quiquampoix, *J. Colloid Interf. Sci.*, **89**, 94 (1994).

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