Bentonite-supported catalase

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Abstract: The properties of the clay bentonite as a support for enzyme immobilization were studied using the enzyme catalase. Such an immobilization does not result in enzyme inactivation and constitutes a valuable method for immobilizing catalase at high ionic strength. The bentonite-supported catalase was characterized in terms of pH and ionic strength dependencies, thermal and storage stability and kinetic parameters. These studies indicate that bentonite is a valuable support for the simple adsorption of enzymes.

Keywords: bentonite, catalase, immobilization.

INTRODUCTION

Bentonite is a clay which has the highest absorbent capacity of any mineral clay, as well as a high adsorptive capacity.[1] Its advantages over other minerals, especially with regards to its low density and high specific surface, have been accepted for a long time, and they account for most of the current uses of bentonite.[2] In the literature, Naidja and Huang (1996) immobilized the enzyme aspartase on clays to determine aspartic acid in various media,[3] while Dong *et al*. (1995) made a kinetic analysis of clays modified with enzymes.[4] Futhermore, *et al*. (1994) studied the adsorption of certain proteins, obtained by fermentation, on silica gels.[5] In the recent years, enzymes have been immobilized by hydrophobic association.[6] Bentonites possess important and unique properties which give them great commercial value in decolorizing oils, in the manufacture of catalysts, in bonding of molding sands, in the preparation of oil-well drilling muds, and in many other relatively minor uses. However, some examples of pertinent correlations between properties and composition can be given. It was, therefore, thought worthwhile to study the ability of bentonite to immobilize enzymes without covalent attachment, taking advantage of its adsorptive properties. This note reports the preparation and characterization of bentonite-supported catalase. Recently, various immobilization of enzymes on polymers have appeared in the literature.[7] In addition,

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Shimomura *et al.* (1994) investigated the adsorption of the enzyme lipase on silica gel.[8] Tang *et al.* (1993) studied the adsorption of alkaline phosphatase on bentonite.[9]

EXPERIMENTAL

Solid support

Wet micronized bentonite was supplied by Kütahya, Turkey). In order to prepare the support, the bentonite was dispersed, by vigorous stirring for 30 min, in an equilibration buffer (0.05 M sodium phosphate buffer, pH 7.0) and further centrifuged. This treatment was repeated several times until a constant pH was obtained. Bentonite suspensions were prepared at 25 mg/ml. These suspensions exhibited considerable stability, with no sedimentation after 24 h. In this study for standard purposes, solutions of catalase (0.01 mg ml^{-1}) in 0.05 M sodium phosphate buffer, pH 7.0 were added to 25 mg ml^{-1} suspensions of Kütahya bentonite, in a 1:4 volume ratio. The catalase-clay mixtures were incubated at 30 ºC for 60 min with gentle shaking. The enzyme-bentonite complex was removed by centrifugation at 3000 g for 15 min and then washed 4 times with 5 ml of phosphate buffer. The amount of enzyme activity adsorbed on the clay was calculated as the difference between the values obtained in the original preparation and the supernatants.

Enzyme

Catalase (hydrogen peroxide oxidoreductase; EC 1.11.1.6) from bovine liver, glutaraldehyde, glyoxal (trimer; dihydrate) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Hydrogen Peroxide, tetrasodium pyrophosphate and all other chemicals were obtained from Merck AG (Darmstadt, Germany). Catalase catalyzes the reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$
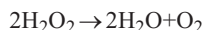
Since the reaction is first-order, the amount of peroxide substrate decomposed is directly proportional to:

1-the concentration of the substrate
2-the concentration of the enzyme.

The assay conditions must be identical to compare the activities of various preparations. The H_2O_2 concentrations from the start to the finish of the assay must be accurately defined. These conditions are not met by most published procedures.

Catalase assay

The activity of free and immobilized catalase were determined spectrophotometrically by the direct measurement of the decrease of light absorption at 240 nm caused by the decomposition of hydrogen peroxide by the enzyme. The activities were measured under optimum conditions. Approximately 0.5 mg of catalase immobilized bentonite was mixed with 2.9 ml H_2O_2 solution in 0.05M phosphate buffer (pH 7.0) at 30 ºC. The immobilized enzyme used throughout the present study necessitated a 5- fold final dilution of the samples. Thus, the turbidity due to the suspended clay in spectrophotometric assays was negligible. These activity assays were carried out over the pH range of 4.0 – 9.0 and temperature range of 10–70 °C to determine the pH and temperature profiles for the free and immobilized enzyme. The catalase-bentonite suspensions were lyophilized and stored at 2 ºC. They could be regenerated by shaking the lyophilized material in a suitable volume of glass-distilled water.

RESULTS AND DISCUSSION

Effect of pH on enzyme activity

Free and immobilized catalase preparations were incubated at 30 ºC, for 24 h at different pH in appropriate 0.05 M phosphate buffer pH 5.0-8.0 and the relative

activity was measured under standard conditions. The optimum pH for free catalase was 7.0, Fig. 1, whereas for the supported catalase it was 8.0. This shift in optimum pH value may be interpreted as resulting from enzyme binding to positive charge regions of the clay. Moreover, this shift in the optimum pH was observed in spite of the high ionic strength (0.05 M) in the assay mixture, where minimization of the electrostatic effect would be expected.
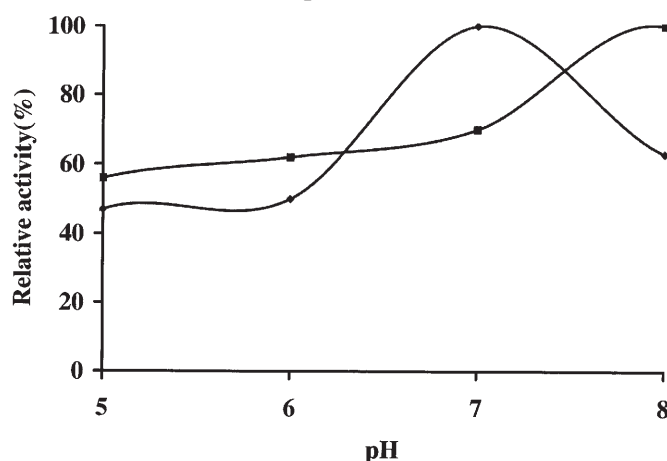

Fig. 1. Effect of pH on the enzyme activity of (■) bentonite-supported catalase and (◆) free catalase. Different phosphate buffers were used, whereby the ionic strength was maintained constant at 0.05M.

Effect of ionic strength on enzyme activity

The results obtained for both free and supported catalase are given in Fig. 2. The bentonite-supported catalase appears significantly more active at high ionic strengths than the free enzyme. Hence, the adsorption of catalase gives additional stabilization to the active site against electrostatic interactions. The influence of ionic strength on bound and free enzyme was determined by adding 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 M sodium phosphate buffers to the reaction medium at constant pH value. These results show that when the buffer concentration (*i.e.*, ionic strength) was increased, the activity of the immobilized catalase was more affected than the activity of the free catalase.

Effect of temperature on the activity and thermal stability of the enzyme

Thermal stability studies of free and immobilized catalase were performed by measuring the residual activity of the enzyme after exposure to three different temperatures (10-70 ºC) in 0.05M phosphate buffer (pH 7.0) for 2 and 5 h. The activity of the samples was determined under optimum conditions. The effect of temperature on the stability of free and immobilized catalase is shown in Fig. 3. The supported enzyme at 50 ºC shows a higher activity than the free enzyme. At 60 ºC,
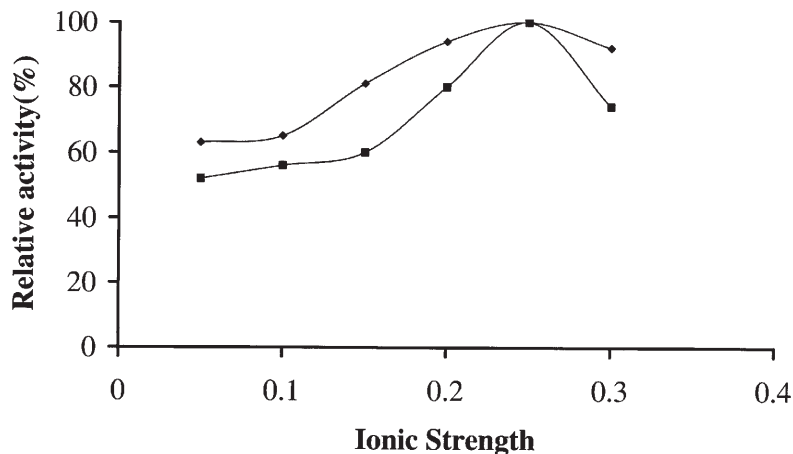
Fig. 2. Effect of ionic strength on the activity of (■) bentonite-supported catalase and (♦) free catalase. This study was performed by varying the concentration of the assay phosphate buffer (pH 7.0).

both the supported and the free enzymes undergo decomposition of structure. The bentonite-supported catalase shows a higher optimum activation temperature than the free catalase, as well as a greater degree of activation. The thermal stability of the supported catalase is also increased.

Kinetic parameters

Apparent Michaelis constants and maximum rate values for free and supported catalase were calculated from the slope of the plot of the Michaelis-Menten equation, according to the method of Wilkinson modified by Cornish-Bowden.[10] The Lineweaver-Burk plots of the experimental values are shown in Fig. 4, in com-
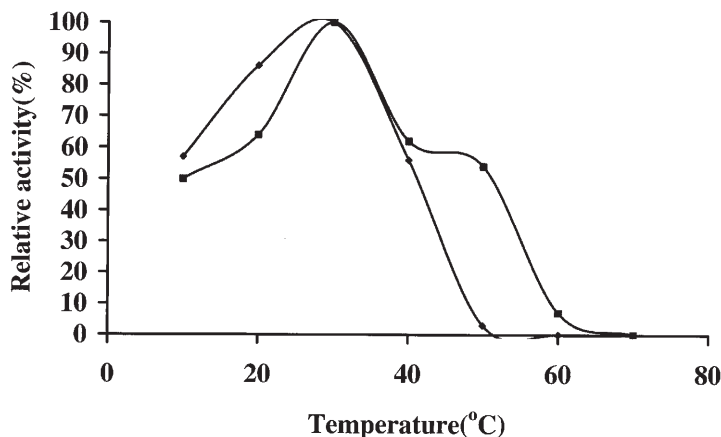

Fig. 3. Effect of temperature on the activity of (■) bentonite-supported calatase and (♦) free catalase. Each activity value was obtained after 15 min incubation at the selected temperature and is expressed as a percentage (pH 7.0).

$$y = 0.2368x - 0.0179$$
$$R^2 = 0.9796$$

a)

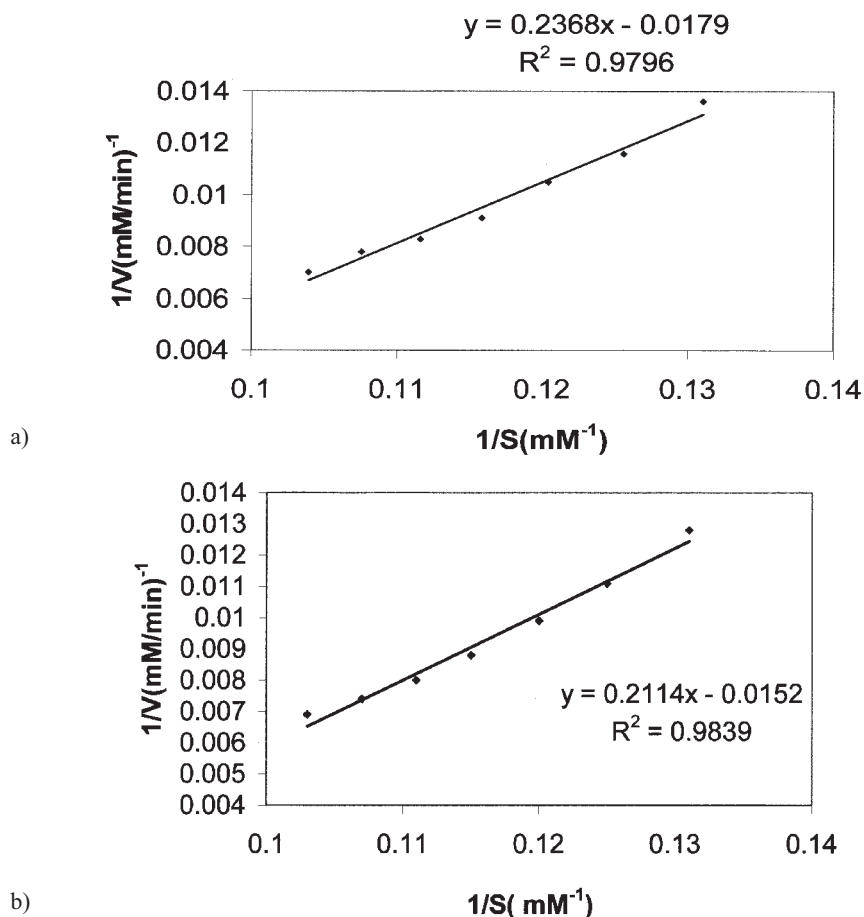$$y = 0.2114x - 0.0152$$
$$R^2 = 0.9839$$

b)

Fig. 4. Lineweaver-Burk plots, (a) free catalase and (b) immobilized catalase. Reactions were carried out in 0.05M phosphate buffer pH 7.0.

parison 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 microenvironmental effects on the adsorbed enzyme and an unaltered mechanism of catalysis. This observation also indicates that there is no local increase of the catalase concentration which would result in a decrease of the apparent K_m value. The enzyme activity was determined using substrate solutions in different concentrations to obtain the $1/S$ and $1/V$ values. The K_m and V_{max} values were determined by means of the Lineweaver–Burk plot. The Michaelis-Menten equation is written and correlated to determine the K_m and V_{max}.

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

The value of K_m was found to be 13.90 mM whereas the V_{max} was calculated as 65.78 μM/min for free catalase. The K_m value was found to be 13.22 mM and the V_{max} value was found to be 55.86 μM/min for the immobilized catalase.

The development of versatile supports constitutes one of the aspects which deserves more attention among workers in the field of enzyme immobilization. The nature of the carrier selected influences for immobilization method. In the present case it is easy and cheap and results in only a low degree of enzyme inactivation. Thus, it is evident that inexpensive solid supports with high adsorptive capacity should have considerable utility as enzyme carries. Other properties, such as high rigidity and lack of deformation, are additional advantages of inorganic carriers as enzyme supports.

ИЗВОД

КАТАЛАЗА НА БЕНТОНИТУ КАО НОСАЧУ

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Проучаване су особине бентонитне глине као носача имобилизованих ензима на примеру ензима каталазе. Оваква имобилизација не доводи до инактивације ензима и представља ваљан метод имобилизације каталазе у растворима високе јонске јачине. Каталаза на бентониту као носачу испитана је у односу на зависност од pH и јонске јачине, термичке и временске стабилности, а одређени су и кинетички параметри. Ова проучавања показала су да је бентонит ваљана подлога за једноставну адсорпцију ензима.

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REFERENCES

1. R. H. S. Robertson, *Chem. Ind.* **5** (1957) 1492
2. R. H. S. Robertson, *Silicates Ind.* **38** (1973) 33
3. A. Naidja, P. M. Huang, *J. Mol. Catal. A-Chemical.* **3** (1996) 106
4. X. D. Dong, J. J. Cha, *J. Electroanal. Chem.* **2** (1995) 201
5. Ito, M. Yamauchi, *Collloids and Surfaces.* **74** (1993) 1
6. S. Staunton, H. Quiquampoix, *J. Colloid Interface Sci.* **1** (1994) 89
7. S. Sonnet, E. Philip, Mc. Neill, P. Gerald, *J. Am. Oil Chem. Soc.* **12** (1994) 1421
8. M. Shimamura, H. Kikuchi, H. Matsumoto, T. Yamauchi, S. Miyauchi, *Polymer J.* **27** (1995) 974
9. Tang, Xiaozhong, Shen, Y. Fei, L. Steven, *Microporous Materials* **71** (1993) 65
10. A. Cornis-Bowden, *Principles of Enzyme Kinetics*, Butterworths, London, 1976, p. 142.