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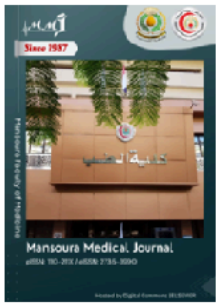

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THERMAL DEACTIVATION OF IMMOBILIZED CATALASE

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INTRODUCTION

The rates of enzyme catalyzed reactions typically increase in Arrhenius fashion near the enzyme natural temperature, but increasing temperature eventually results in rapid decline in enzyme activity. Thermal deactivation of enzyme may involve both reversible and irreversible processes. When the temperature is kept constant,a number of enzyme systems are found to be irreversibly denatured with time, often following approximately a first-order decay law(I). If the heat stability of an enzyme is enhanced by immobilization, the potential utilization of such enzymes will be extensive(2,3). In this work the thermal deactivation rate measurements were reported for both the free and immobilized forms of catalase.

MATERIALS AND METHODS

Crystalline Bovine liver catalase was purchased from Sigma Chemical Company. The enzyme was immobilized on nonporous glass beads using the method of Malikkides and Weiland(4),catalase activity was determined using the oxygen electrode(S). The deactivation experiments were carried out in 150 ml stoppered flasks. Temperatures in the range from 60-70 were maintained constant and the enzyme was exposed for periods up to 50min. Catalase was placed in citrate phosphate buffer (pH 7) in the absence of hydrogen peroxide, then assayed for remaining activity at 25°C.

Deactivation is first order if :

$$dE/dt = - k_d E \qquad (I)$$

which in integrated form is:

$$\ln E(t)/E(o) = - k_d t \qquad (2)$$

where k_d is the denaturation rate constant(thermal deactivation rate constant) and E is the enzyme activity in IU/g of support for the immobilized enzyme or IU/ml of solution for the soluble form.The plot of ln $E_{(t)}/E_{(o)}$ versus time gives a straight line from which the denaTuration rate constant can be determined.The suggestion that the deactivation behavior obeys the Arrhenius Kinetics was also tested. This means that the temperature dependence of kd is given by :

$$kd = A_{exp} (-E_d/RT) \quad (3)$$

where E_d is the deactivation energy, A is the frequency factor, R is universal gas constant and T is the absolute temperature. The logarithmic form of equation (3) is :

$$\ln kd = - E_d/R.1/T + \ln A \quad (4)$$

A plot of ln k_d versus 1/T yields a straight line with a slope equal to $-E_d/R$ from which the deactivation energy can be calculated.

RESULTS

A plot of ln E(t)/E(o) % versus time for the immobilized enzyme is shown in Fig. (1). The remaining enzyme activity in all cases was the mean of three measurements with a maximum standard deviation of (± 5%). The deactivation of soluble and immobilized forms is compared in Fig.(2) for a temperature of 62°C. The rate constants for deactivation for both forms are shown in Table (1). In Fig. (3) the rate constants of Table (I)are plotted in the form suggested by equation (4).

DISCUSSION

The catalytic activity of enzymes increases with temperature as in case of usual chemical catalysis. However as enzymes consist of protein and are generally unstable to heat, the enzyme reaction cannot be practically carried out at high temperature. The heat stability of many immobilized enzyme has been studied, and there are examples shwoing increases, no change and decreases of heat stability on immobilization.

In the present results, the first order behavior is immediately apparent. This is in agreement with the observation of Wasserman et al., (6), and Yamamoto et al(7). The Immobilized enzyme showed reduced sensitivity to thermal deactivation over the soluble form. Enhancement of heat stability by immobilization has been observed for many enzymes, but no correlation between heat stability and immobilization method has been established(8,910°).

Chibeta(ll) found that the heat stability of mold aminoacylase immobilized by ionic binding with DEAE-Sephadex, alkylation with iodoacetyl cellulose and entrapping with polyacrylamide gel increased considerably compared with that of the native enzyme. The soluble aminoacylase was almost completely inactivated by heat treatment at 70°C for 10 min, while the remaining activities of DEAE-Sephadex-aminoacylase, iodoacetyl cellulose-aminoacylase and polyacrylamide gelaminoacylase after similar treatments were 90%, 50% and 30% respectively.Enhancement of heat stability was also observed in pronases immobilized by using ethylene-malic anhydride copolymer and CNBr-activated cellulose[12] and acid phosphatase immobilized by carrier cross-linked with glutaraldhyde and polyacrylamide gel[13]. Horigome et al[14], suggested that the heat stability of immobilized enzyme depends on the Pore size of the carrier used for immobilization. On the other hand, the remaining activity of invertase immobilized by ionic binding with DEAE cellulose after treatment at S0°C for 30 min was 40% while the native enzyme was 100% after similar treatment[15].

The results shown in Fig. (3) indicate that Arrhenius Kinetics well represent the deactivation behavior. This finding further recommends the work of Yamamoto et al(7),who showed that the thermal deactivation rate constant can be expressed by an Arrhenius-type equation.The deactivation energy of the soluble catalase is found to exceed that of the immobilized form indicating that the rate at which the soluble enzyme is denatured is a slightly stronger function of temperature.The deactivation energies for both forms are much higher than generally found for most chemical reactions reflecting the thermal sensitivity of catalase.

SUMMARY

The thermal stability of catalase immobilized by covalent coupling was determined and was compared to that for the soluble enzyme. The results indictaed that immobilization of catalase resulted in improved resistance to thermal deactivation and that the deactivation energy of the soluble enzyme exceeds that of the immobilized form.

Table (1) : Thermal deactivation rate constants of catalase

Temperature	Soluble (min^{-1})	Immobilized (min^{-1})
60	0.016	0.012
62	0.025	0.017
64	0.072	0.031
66	0.129	0.057
68	0.215	0.096
70	0.316	0.168

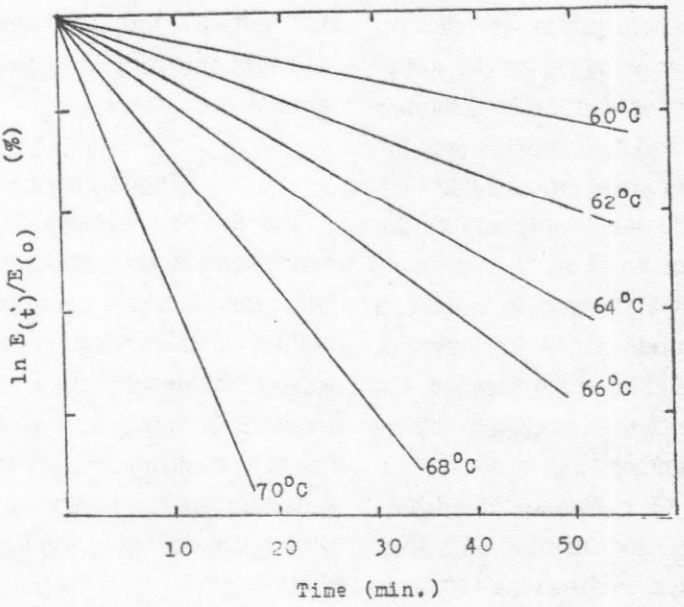

Fig. (1): ln remaining activity of immobilized enzyme as a function of time at various temperatures.

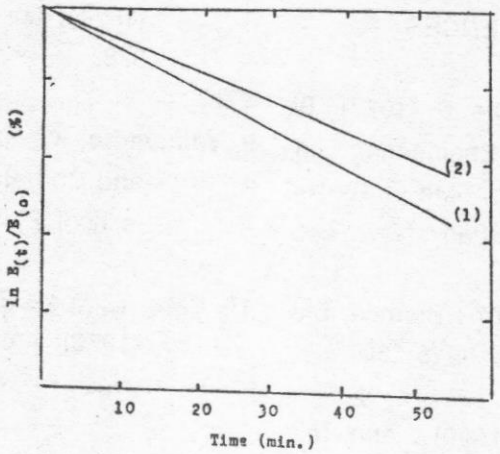

Fig. (2): Comparison between Soluble (1) and Immobilized (2) enzyme deactivation at 62°C.

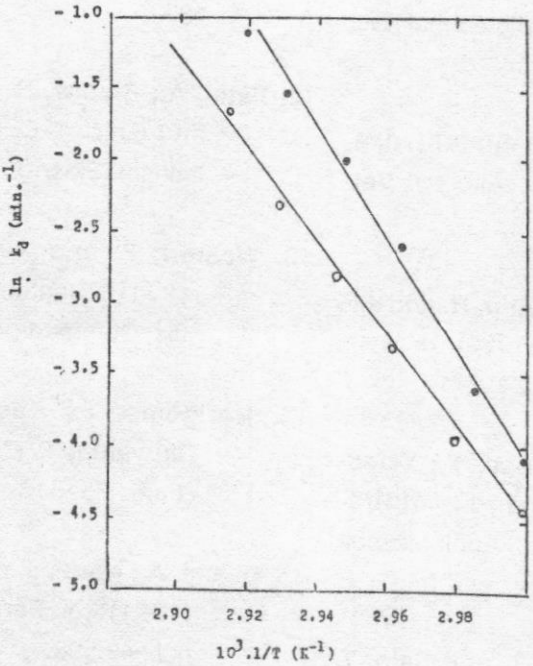

Fig. (3): Arrhenius plot for soluble(•—•) and immobilized (o—o) catalase.

E_d for soluble enzyme = 72.6 K cal/mol
E_d for immobilized enzyme = 61.8 K cal/mol

REFERENCES

1. **Bailey, J. and Ollis, D. (1977) :** Biochemical Engineering Fundamentals, Mc-Graw-Hill Book Comapany, New York.

2. **Tarhan, L. (1990) :** Biomed. Biochem. Acta. 49 (5): 307.

3. **Nilsson, X.G. (1990) :** Ann. N.Y. Acad. Sci., 613:431.

4. **Malikkides, C.O and Weiland, R.H. (1982):** Biotechnol. Bioengin., 24, 2414.

5. **Mostafa, M.A. and Abdel-Halim, S.A. (1985):** Alex. J. Vet. Sci. Vol 1 No 2.

6. **Wasserman, B., Hultin, H. and Jacobson, B. (1980):** Biotechnol. Bioeng., 22: 271.

7. **Yamamoto, K.; Tosa, T.; Yamashita,K. and chibata,1.(1976):** J. Appl. Microbiol, 3: 169.

8. **Chibata, I., Tosa, T. and Sato, T. (1974):** Appl. Microbiol., 27: 878.

9. **Yamamoto, K., Sato, T., Tosa, T. and Chibata, I. (1974):** Biotechnol. Bioeng. 16: 1589.

10. **Sato, T., Tosa, T. and Chibata, I (1976):** Arch. Biochem. Biopygs., 147:788.

11. **Chibata, I. (1978):** Immobilized enzymes, John Wiley and Sons, New York London, p. 132.

12. **Patel, A.; Stasiw, R.; Broun, H. and Ghiron, C. (1972):** Biotechnol. Bioeng., 14: 1031.

13. **Weston, P. and Avrameas, S (1971):** Biochem. Biophys. Res. Commun., 45: 1574.

14. **Horigome, T.; Kasai, H. and Okuyama, T. (1974):** J. Biochem., 75: 513.

15. **Usami, A.; Noda, J. and Goto, K. (1971):** J. Ferment. Technol., 49:598.

التثبيط الحرارى لانزيم الكاتابيز المثبت

ملخص عربى

تم دراسة تأثير الحرارة على فقدان انزيم الكاتاليز المثبت لبعض نشاطه والمقارنة بينه وبين الانزيم الحر. وقد أظهرت الدراسة أن عملية تثبيت الكاتاليز قد زادت مقاومة الانزيم لدرجات الحرارة المرتفعة حيث احتفظ بنسبة أعلى من النشاط عن الانزيم الغير مثبت.

كما أثبتت الدراسة زيادة طاقة التثبيط الحرارى للانزيم الحر عنها فى الانزيم المثبت.