

THERMAL ACTIVATION OF IMMOBILIZED PAPAIN*

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Abstract Papain (Papainase, EC 3.4.22.2) was immobilized on porous silica beads by cross linking with glutaraldehyde. The thermal activation of this immobilized papain in aqueous system was found at a temperature range from 50 to 90°C. The higher the temperature, the more active the immobilized papain will possess. At the same time, the durability of the immobilized papain on heating was greatly improved. The effect of additives and salts on the activity of the immobilized papain were also studied. The results showed that the additives and some of the salts studied could markedly enhance the activity of the immobilized papain at elevated temperature.

Keywords Papain; Immobilization; Thermal activation; Thermal stability

INTRODUCTION

In most cases, enzymatic industrial processes are carried out at elevated temperature. Under this condition, the rates of enzymatic reaction generally are accelerated, the likelihood of bacterial contamination can be sharply reduced, and the productivity can be improved because solubility of most substrates increases with temperature^[1].

In recent years, much efforts has been focused on the improvement of thermal stability of enzymes in aqueous systems^[2~6]. One of the major methods to improve enzyme thermal stability is via enzyme immobilization^[1].

Papain (Papainase, EC 3.4.22.2) is abundant in natural resources, and can hydrolyze lots of proteins and peptides. Papain is often used in scientific research and usually employed in medicine, industries and the production of pharmaceuticals. In our previous paper we reported the immobilization of chymotrypsin (EC 3.4.21.1) on porous silica beads. In the present paper, we wish to report a new finding on the thermal activation and thermal stability of immobilized papain.

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EXPERIMENTAL

Materials

Papain was purchased from Fluka (Switzerland) and used without further purification. Porous silica bead (120~160 mesh) was obtained from the Second Tianjin Chemical Plant. And glutaraldehyde (25% (W/V)) was from Merck. 3-Aminopropyltriethoxysilane was obtained from Chemical Plant of Wuhan University and redistilled before use (b.p. 213~216°C). Cysteine and casein were biochemicals. All other reagents used in this study were of analytical grade.

Enzyme immobilization

Porous silica beads were treated with acid and followed by reacting with 3-aminopropyltriethoxysilane^[7]. Papain was immobilized on the functional porous silica beads by cross linking with glutaraldehyde for 12 h at 25°C and then washed with redistilled water. Immobilized papain was dried in vacuo at 25°C. Enzymatic activity recovery was 40%.

Enzymatic activity assay

The standard enzymatic activity assay was carried out for 30 min at 37°C according to the procedure of Weetall and Mason^[8] by recording the absorbance at 280 nm in Shimadzu UV-240 spectrophotometer.

Thermal activation studies

The tests of temperature effects on the activity of immobilized papain were carried out by preincubating the immobilized enzyme in aqueous systems with different salts for 1 h at 50, 60, 70, 80 and 90°C, respectively. After cooling in ice, substrate, casein, was added and followed by enzymatic activity assay. Control sample was dissolved in redistilled water and not preincubated. The concentration of salts were 0.1 mol·dm⁻³ and was adjusted to pH 7.0 with sodium hydroxide or hydrochloric acid solution.

Effects of duration of heating on the activity of immobilized papain were investigated by preincubating the immobilized papain at 70,80,90°C for different period of time. After cooling in ice, the enzymatic activity assays were carried out as mentioned above.

The additives, cysteine and EDTA (disodium salt), were used to study the effects on the activity of the enzyme. The experimental conditions were the same as described above.

RESULTS AND DISCUSSION

Temperature effects on the activity of immobilized papain

It is well known that the thermal stability of an enzyme can be improved via its immobilization^[8~10]. For example, free papain can retain its original enzymatic activity only at an incubation temperature below 35°C, whereas immobilized papain can be incubated even up to 60°C without losing its activity. However, in our study on the activity and thermal stability of papain immobilized on porous silica beads, we found that in the aqueous system, the immobilized papain still retain its activity, but also gradually increased even up to a temperature of 90°C. We also studied the influences of temperature change on the enzymatic activity by the addition of different salts in aqueous systems. The enzymatic activity of immobilized papain reached to a relatively high level up to 90°C in the case of sodium phosphate, ammonia sulfate and acetate system (shown in Fig. 1).

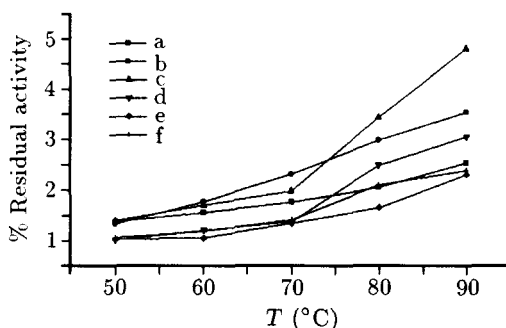


Fig. 1 Temperature effects on the activity of immobilized papain. Preincubation of 0.1 g of the immobilized papain from 50 to 90°C for 1 h was carried out in different aqueous systems: calcium chloride solution (a), ammonia sulfate solution (b), sodium phosphate solution (c), sodium acetate solution (d), sodium chloride solution (e), and redistilled water (f)

Effects of duration of heating on the activity of immobilized papain

Fig. 1 has shown that by heating at high temperatures for 1 h, the immobilized papain is activated and the activity of the immobilized papain increases as temperature is raised from 50 to 90°C. It would seem necessary to further study the relationship between thermal activation and the duration of heating. Results of these experiments were shown in Fig. 2, by plotting the residual activity of the immobilized papain versus the duration of heating at 70°C (Fig. 2A), 80°C (Fig. 2B) and 90°C (Fig. 2C), respectively. It can be seen that the enzymatic activity could retain at a high level after a given time of heating.

We also studied the effects of duration of heating on the activity of the immobilized papain in the presence of different kinds of salts in aqueous systems. The overall tendency of changes was similar.

Fig. 1 and Fig. 2 show that the immobilized papain on porous silica beads can be activated by heating. After heating, the immobilized papain could probably be changed to the most favorable conformation; while the diffusional limitations might be reduced. Thus, the immobilized papain possessed the higher activity. Nevertheless, the effectiveness of thermal activation is also related to the carriers, the nature of enzymes, the methods of immobilization and the medium as well. Yet, the mechanism of thermal activation remains to be elucidated.

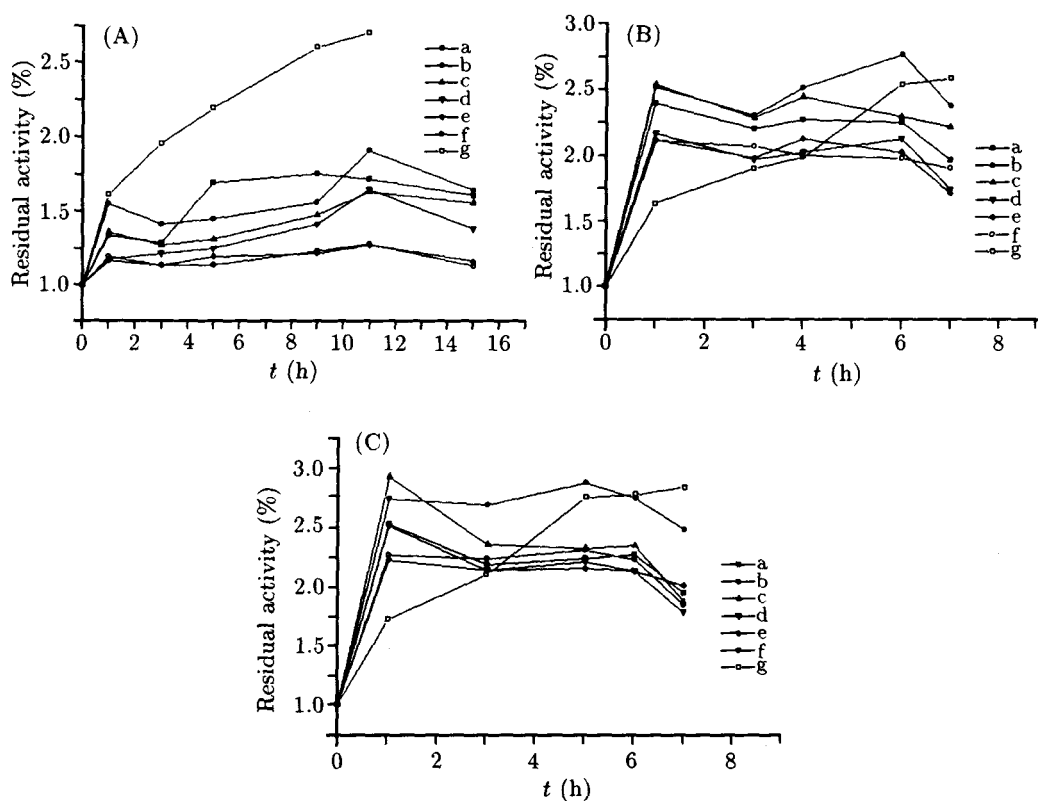


Fig. 2 At 70°C (A), 80°C (B), and 90°C (C), thermal activation were studied in different aqueous systems: calcium chloride solution (a), ammonia sulfate solution (b), sodium phosphate solution (c), sodium acetate solution (d), sodium chloride solution (e), redistilled water (f), sodium phosphate solution including 8 mmol cm⁻³ Cysteine and 1 mmol dm⁻³ EDTA (g)

Additives effects on the activity of immobilized papain

It is known that EDTA can bind metal impurities which are strongly inhibitory,

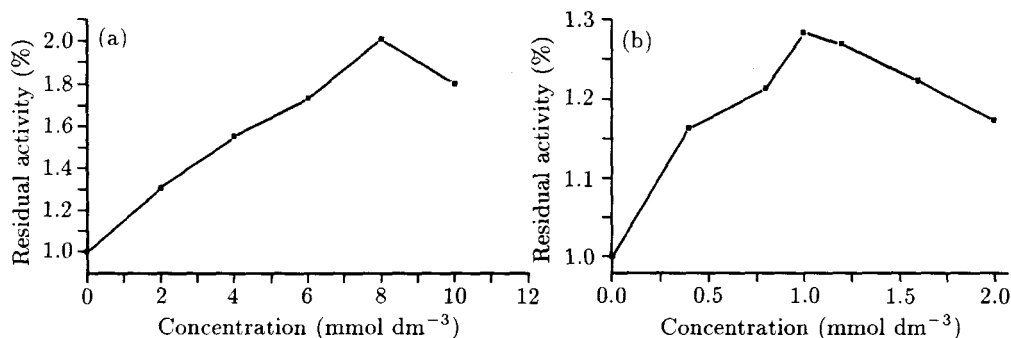


Fig. 3 Influence of concentration of additives on the activity of the immobilized papain: Cysteine (a) and EDTA (b)

and cysteine is a reducing agent which can prevent the thiol group of the active-site of papain from being oxidized. Fig. 3 shows the activity increasing of the immobilized papain caused by the addition of cysteine (a) and EDTA (b) with a definite concentration. Fig. 2 (A, B, C) illustrates the immobilized papain could be more effectively activated with the addition of both cysteine and EDTA in aqueous systems. In addition, the enzymatic activity still increased even if the immobilized papain was incubated for 7 h at 80°C or 90°C.

REFERENCES

- [1] Klivanov, A.M., in "Advances in Application Microbiology", Ed. by Laskin, A.I., Academic Press, New York, **1983**, No. 29, p.1
- [2] Skerker, P.S., Clark, D.S., *Biotechnol. Bioeng.*, **1989**, 33, 62
- [3] Tomazic, S.J., Klivanov, A.M., *J. Biol. Chem.*, **1988**, 263, 3086
- [4] Zale, S.E., Klivanov, A.M., *Biochemistry*, **1986**, 25, 5432
- [5] Volkin, D.B., Klivanov, A.M., *Biotechnol. Bioeng.*, **1989**, 33, 1104
- [6] Volkin, D.B., Klivanov, A.M., *J. Biol. Chem.*, **1987**, 262, 2945
- [7] Liu, L.J., Zhuo, R.X., *Ion Exchange and Adsorption*, **1995**, 1, 541
- [8] Weetall, H.H., Mason, R.D., *Biotechnol. Bioeng.*, **1973**, 15, 455
- [9] Hayashi, T., Hirayama, C., Iwatsuki, M., *J. Appl. Polym. Sci.*, **1992**, 44, 143
- [10] Hayashi, T., Ikada, Y., *J. Appl. Polym. Sci.*, **1991**, 42, 85