

IMMOBILIZATION OF GLUCOSE OXIDASE AND CELLULASE BY CHITOSAN—POLYACRYLIC ACID COMPLEX

WANG Lingzhi (王玲治) and JIANG Yingyan (江英彦)

(*Institute of Chemistry, Academia Sinica, Beijing*)

ZHANG Changde (张长德) and HUANG Dexiu (黄德琇)

(*Xi'an Jiaotong University, Xi'an, China*)

ABSTRACT

This study is concerned with chitosan—polyacrylic acid complex as a carrier to immobilize glucose oxidase (GOD) and cellulase. The optimum temperature of the immobilized GOD (IG) was determined to be 60 °C which is higher than that of the native GOD about 40 °C. The optimum temperature of the immobilized cellulase (IC) was determined to be about 30 °C higher than that of native cellulase. Both of the optimum pH of IG and IC shifted one pH unit to acid. Immobilized enzyme may be used in more wide pH range. Their storage life are much longer compared with their native states. Both of them can be reused at least 12 times.

INTRODUCTION

Enzymes are efficient and selective catalysts, which can catalyze various biochemical reaction in living body at mild conditions. But their native states are very sensitive to the change of environment, difficult to be separated from substrates and products. They can be used only once and some enzymes are expensive. However, in enzymes immobilization there are some advantages such as the easiness be separated, the possibility of continuous operation and the effective use of the enzyme and so on.

Recently a novel immobilization method of enzymes by use of polymer—polymer complexes^[1, 2] have been reported. Chitosan, an abundant natural polymer, has excellent resistance to chemicals, high tissue tolerance and no toxicity^[3]. Chitin and its derivative chitosan feature certain remarkable characteristics that qualify them as good supports for enzyme immobilization^[4, 5, 6], but the times of repeated use of the immobilized enzyme is rather poor.

This paper is concerned with the immobilization of cellulase and glucose oxidase (GOD) by chitosan (CS)—polyacrylic acid (PAA) complexes.

EXPERIMENTAL

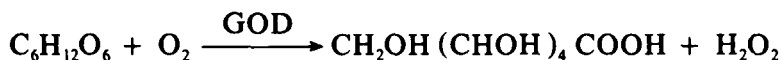
1. Reagents and instrumentation

All reagents used were reagent grade. The—NH₂ content of CS is 8.24%. Cellulase and GOD were purchased from Tokyo Kashi Corporation and Sigma Chemical Company. UV—visible spectra were taken on UVIDEK—320 made in Japan.

2. The enzyme reaction

(1) Oxidation of glucose

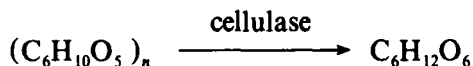
According to the literature^[7,8] the reaction may be expressed in the following scheme:



10mg glucose and CS-GOD-PAA immobilized enzyme (contain 0.5mg GOD) was put into 10mL acetate buffer. This was stirred at room temperature for an hour.

(2) Hydrolysis of cellulose

The procedure of the reaction may be expressed as follows:

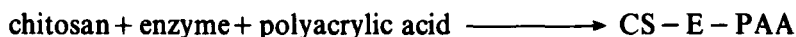


The cotton was pretreated with 50% sulfuric acid. It was precipitated with acetone, filtered, washed with acetone and then dried, the solvable cellulose was obtained.

10mL (108mg/litre) cellulose aqueous solution and CS-cellulase-PAA immobilized enzyme (containing 0.2mg cellulase) was stirred at room temperature for about 24 hours.

3. Preparation of immobilized enzymes

Preparation of CS-E-PAA immobilized enzymes is as follows:



Chitosan was redissolved in 1.5% acetic acid. 5ml 0.04M chitosan solution was mixed with GOD or cellulase solution and stirred for 45 minutes, then 5ml 0.04M polyacrylic acid solution was added and stirred for 1 hour. The immobilized enzyme was precipitated, washed with water and then dried.

4. Measurement of the activity of enzyme

(1) Preparation of reagents:

Reagent I: 1mL of HAc-NaAc buffer pH=5 and 0.025ml 1% o-tolidine alcohol solution were mixed and with to 2.0ml.

Reagent II: 1ml of HAc-NaAc buffer solution pH=5 and 0.025ml 1% o-tolidine alcohol solution and 1mg GOD were mixed, and then diluted to 2.0ml with water.

(2). Measurements

Activity of GOD (or IG)

1ml 1mg/ml glucose solution, 0.05mg GOD (or added IG which contain 0.05mg GOD) in 1ml water, 0.5ml 0.5% KI, 0.5ml 1% $(\text{NH}_4)_2\text{MoO}_4$ solution, 2ml Reagent I and 3ml water were mixed. After stirred for 30 minutes. The absorbance of the product was measured at 620nm.

Activity of cellulase (or IC)

10ml of 0.108mg/ml cellulose solution, 0.1mg cellulase (or IC wich contain 0.1 mg cellulase) in 1ml water were mixed, then the mixture was stirred for 24 hours.

1ml clear reaction product solution was mixed with 0.5ml 0.5% KI solution, 0.5 ml 1% $(\text{NH}_4)_2\text{MoO}_4$ solution, 2ml Reagent II and 3ml water then stirred for 30 minutes and the absorbance was measured at 620nm.

RESULTS AND DISCUSSION

1. The structure of the immobilized enzyme with polymer complex

Polymer complexes have excellent mass transference, selective permeability of water and electrolyte, and biological compatibility. These complexes can be divided into following four classes on the basis of the main interactions: polyelectrolyte complexes based on polyanion and polycation; hydrogen-bonding complexes; stereocomplexes based on isotactic and syndiotactic polymers; and charge transfer complexes based on electron accepting and electron donating. The complex of chitosan and polyacrylic acid is hydrogen-bonding type complexes, the shape of the CS-enzyme-PAA immobilized enzymes is as the form of particle.

The scheme of the immobilized enzyme with polymer complex are shown in Fig.1. The enzyme molecular mainly embedded in the interior of the network between the polymer complexes, and have an interaction with the polymer molecules. The configuration and conformation of the enzymes were not broken, so that the immobilized enzymes have a high activity and stability.

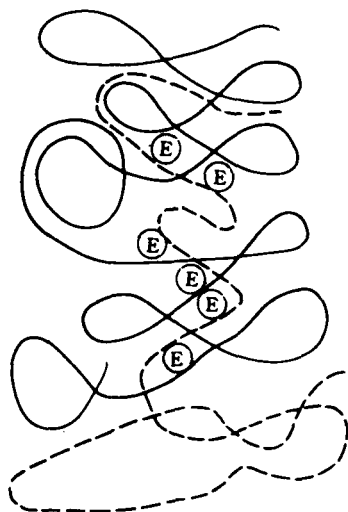


Fig.1 The scheme of the immobilized enzyme with polymer complex

2. The relationship between the activity of enzyme and pH

Figure 2 (a) and (b) show the pH-activity curves of glucose oxidase, cellulase and IG, IC. It is proved that IC can be used in more wide pH range and its resistance to acid and alkali has been improved. The optimum pH of IG and IC have shifted to acid. It is apparent that this change is caused by the change of environment, that is, the enzymes change their environment from the state in vivo to a polymeric heterogeneous matrices. The study of such changes and the factors influencing them are of great interest. It not only assist to know more of the way in which enzyme work in

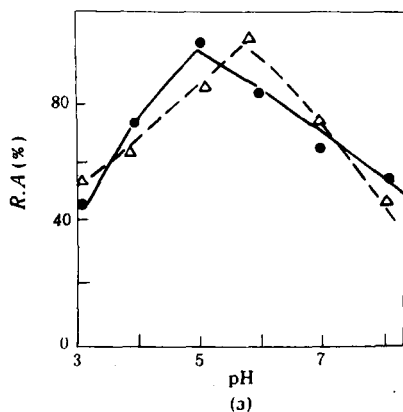


Fig.2 (a) Relationship between GOD activity and pH

--△-- native GOD
—●— CS-GOD-PAA

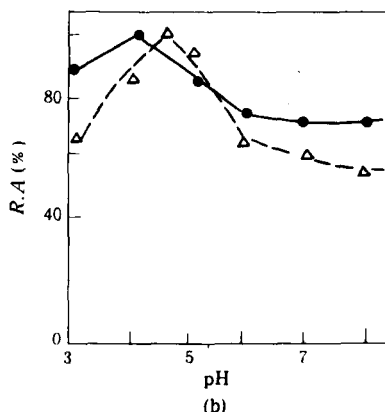


Fig.2 (b) Relationship between cellulase activity and pH

--△-- native cellulase
—●— CS-cellulase-PAA

vivo, but also to be aware of all the factors that may influence the activity of an immobilized enzyme.

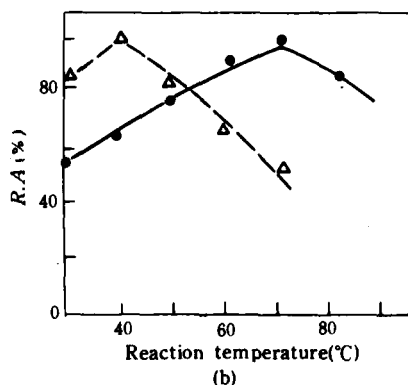
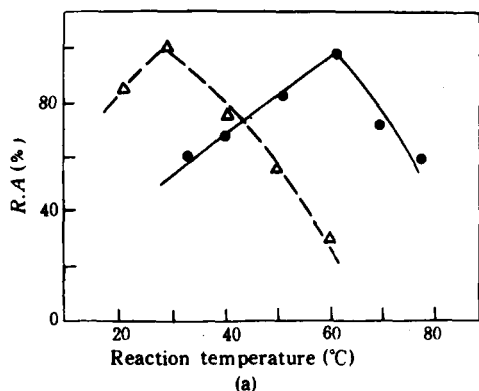


Fig. 3 (a) The relationship between the activity of GOD and the temperature

-- $\triangle$ -- native GOD
—●— CS-GOD-PAA

Fig. 3 (b) The relationship between the activity of cellulase and the temperature

-- $\triangle$ -- native cellulase
—●— CS-cellulase-PAA

R. A stands for relative activity

3. The relationship between the activity of enzymes and temperature

The relationship between the activity of enzymes and temperature are given in Fig. 3 (a) and (b), which indicate the thermal stability of immobilized enzymes, for example, the temperature ranges are 30 °C—60 °C and 40 °C—70 °C for CS-GOD-PAA and CS-cellulase-PAA respectively, is higher than that of native ones.

4. The relationship between the activity of enzymes and reaction time

Fig. 4 (a) and (b) show the relationship between reaction time and glucose oxidation or hydrolysis of cellulose. We can see that the activity of IG is lower than that of the native GOD. However, the final degree of hydrolysis of cellulose by IC is slightly higher than that of the native cellulase. The reason may be due to that the native cellulase has been denatured after prolonged stirring, but the IC can not be denatured because of the increase of its stability by immobilization.

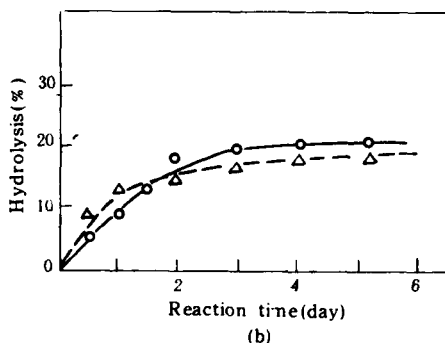
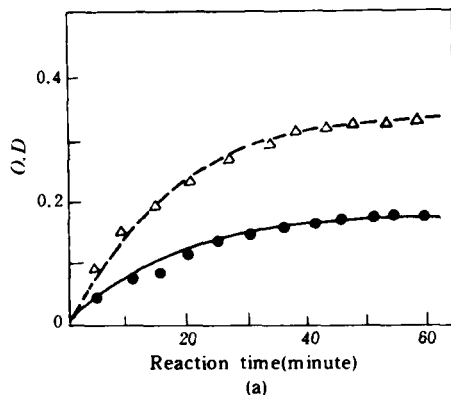


Fig. 4 (a) Effects of reaction time on glucose oxidation rates by IG and native GOD

-- $\triangle$ -- native GOD
—●— CS-GOD-PAA

O. D. stands for optical density

Fig. 4 (b) Effects of reaction time on degrees of cellulose hydrolysis by IC and native cellulase

-- $\triangle$ -- native cellulase
—●— CS-cellulase-PAA

5. The stability of the immobilized enzymes

The main merits of immobilized enzymes are their high specific activity, long life and reproducibility in enzymatic reactions. Figure 5(a) and (b) show the influences of storage stability of the native and immobilized GOD, cellulase respectively at room temperature. It can be seen that GOD keeps only its half activity after stored for 25 days, cellulase for 15 days, but both of the activities of IG and IC have no change after stored for 80 days. The stability of IG and IC have been apparently improved. Figure 6 (a) and (b) show the stability of recycling use of the CS-enzyme-PAA immobilized enzymes. The immobilized enzymes can be reused more than 10 times without any appreciable loss of their relative activity.

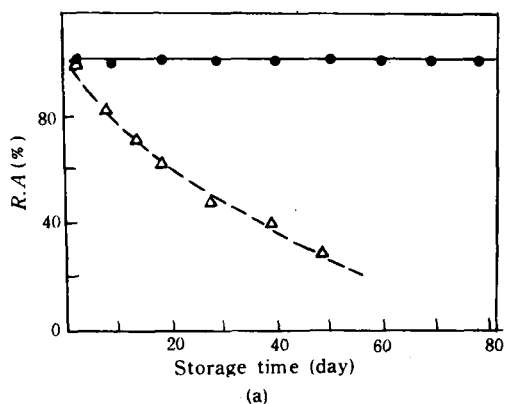


Fig.5 (a) Influences of storage time on the activities of IG and native GOD

--△-- native GOD
--●-- CS-GOD-PAA

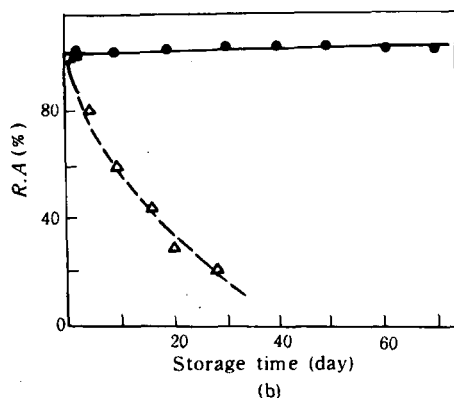


Fig.5 (b) Influences of storage time on the activities of IC and native cellulase

--△-- native cellulase
--●-- CS-cellulase-PAA

under room temperature
R. A stands for relative activity.

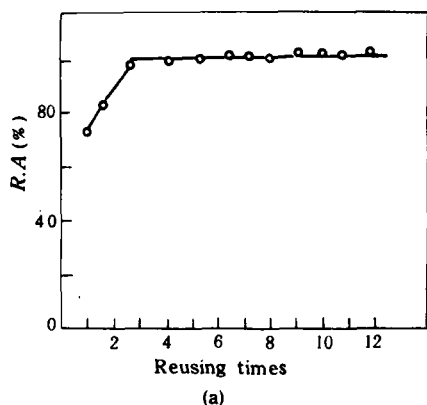


Fig.6 (a) The reusing stability of CS-GOD-PAA

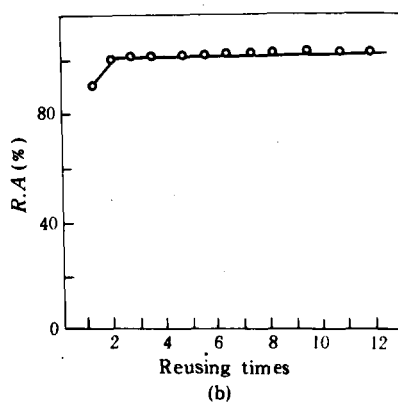


Fig.6 (b) The reusing stability of CS-cellulase-PAA

R. A. stands for relative activity

Keywords :

Immobilized enzyme ; Cellulase ; Glucose oxidase ; Polymer complex

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