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UREASE IMMOBILIZATION INTO POLY(ACRYLAMIDE) - GELATIN GELS

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Abstract- Urease (EC. 3. 5. 1. 5) was immobilized into photographic gelatin-poly(acrylamide) gels by using chromium(III)acetate as crosslinker. The effect of crosslinker concentration , enzyme loading, pH, temperature, supports composition and reuse number on activity were studied. The suitable conditions for an operative and stable system were investigated.

INTRODUCTION

Chemical, medical and waste-processing industries are now using enzymes. Immobilized enzyme usage reduces production expences due to repeated applicability. In recent years semiartificial enzyme systems have been constructed to produce more than one compound[1]

Determination of urea in physiological fluids is of great interest in medicine. Urea is a major metabolic end product, and the removal of its excess has been a major problem[2] for the patients suffering from renal failures.One of the major applications of immobilized urease is the direct removal of urea from blood for detoxification[3], or in the dialysate regeneration systems of the artificial kidney machines[4]. Introduction of urease to the artificial kidney systems reduced the sizes of these machines[1].Piskin et al[5] immobilized urease in bead shaped polymeric gels with radiation technique for use in clinical applications.

Different kinds of solid supports have been used, depending on the method chosen for the attachment of urease, but covalent bonding and microencapsulation techniques have been usually preferred[6]. Because of the existence of reactive groups, hydrophilic polymers were chosen to provide functional groups for coupling with urease. The number of reactive groups can be considerably increased and controlled with such systems[7].

In a previous study of the authors, urease was immobilized into photographic gelatin (PG)[8], on the other hand poly(acrylamide) (pAam) is a well known support material in urease immobilization. In the present work, the mixtures of these two supports with different ratios have been investigated. Chromium(III)acetate (CA) was used as crosslinker.

RESULTS AND DISCUSSION

Chromium(III)sulfate was the second tested crosslinker for urease immobilization. However satisfactory results could not be obtained and further experiments were not performed for this cross linker.

Percent relative activity is plotted versus reuse number for three different support compositions (Figure 1). Weight ratios of poly(acrylamide)/photographic gelatin were 5 for D; 4.2 and 2.5 for C and B respectively. In all compositions, a maximum activity in the range of 75-80% was obtained at the beginning of reuse experiments. After 20 uses, for higher poly(acrylamide) ratios, a decrease in relative activity was observed. This decrease was ca. 50% for D and ca. 40% for C. Maximum enzymatic activity was achieved for the lowest poly(acrylamide) concentration (Figure 1 curve B) of 71% after 20 uses. In all immobilizations, CA concentration was kept constant as 0.08M.

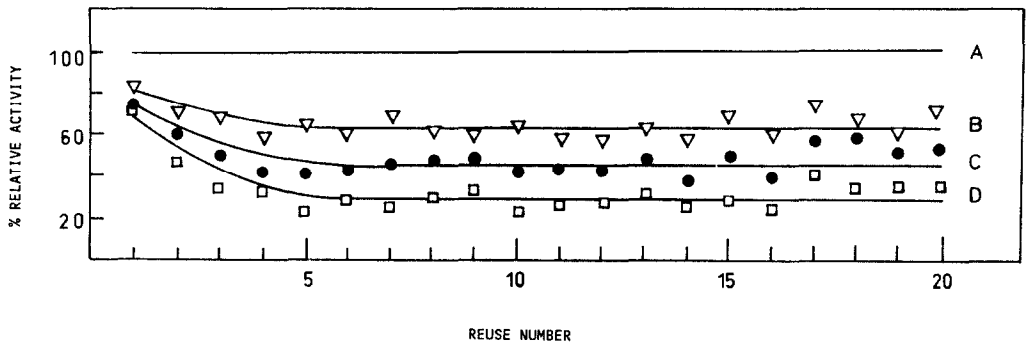

Figure 1 The effect of support ratio on free and immobilized urease activity; poly(acrylamide)/gelatin ratios are 2.5, 4.2 and 5.0 for B, C, and D respectively(A: Free urease relative activity:100; Chromium acetate concentration: 0.08M)

In all immobilization procedures, a decrease in enzymatic activity is seen. In general, immobilization decreases transient time[9] or increases steady-state rate; also enhances the feed-back inhibition by the end product[10].

% Relative activities were calculated according to following formula;

ra:[activity of complex/(total activity of free enzyme used for coupling - activity loss by enzyme leakage)]

ma:maximum value of ra in series of experiments

% Relative activity: ra x 100 % Maximum activity: ra x 100 / ma

In order to observe the effect of urease loading on maximum activity during immobilization, enzyme - poly(acrylamide) / gelatin films were prepared with different urease concentrations (4-20 U/ml). It was observed that with the increasing enzyme concentrations, the activities increased, however maximum activities decreased. The high enzyme concentration in the immobilization mixture favors such inter and intramolecular reactions of the protein which block the active sites of enzyme resulting in a decline of the relative activity.

Slightly higher maximum activities were obtained with CA concentration of 0.05M than 0.08M as enzyme concentration increased (Figure 2). Since % maximum activity decreased with both CA concentrations other cross linker concentrations were not studied.

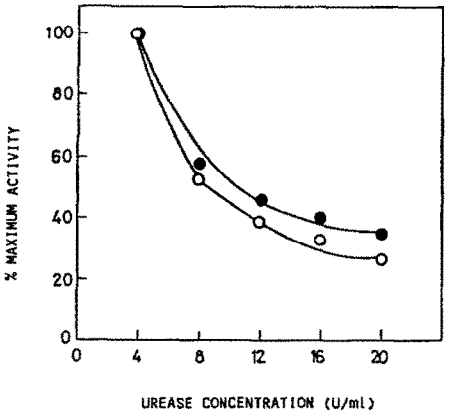

Figure 2 The effect of enzyme loading on immobilized urease activity; poly(acrylamide)/gelatin ratio: 2.5; chromium acetate concentrations are ○ 0.08M and ● 0.05M

The effect of pH on free and immobilized urease is given on Figure 3. The maximum enzymatic activities of free and immobilized ureases were detected at about pH 8, and immobilization improved pH stability considerably.

Optimum temperatures for urea hydrolysis were investigated by changing the incubation temperature from 15° C to 85° C (Figure 4). Maximum activity was obtained at 52° C for immobilized urease. The thermal stability of immobilized enzyme did not differ much from native enzyme.

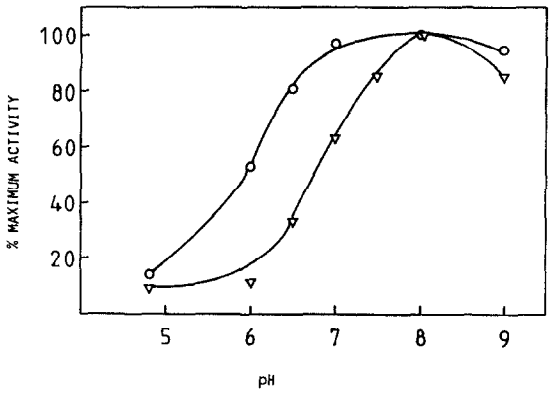

Figure 3 The effect of pH on free and immobilized urease activity; poly(acrylamide/gelatin ratio: 2.5; ○ 0.08M chromium acetate type urease; ▽ free urease

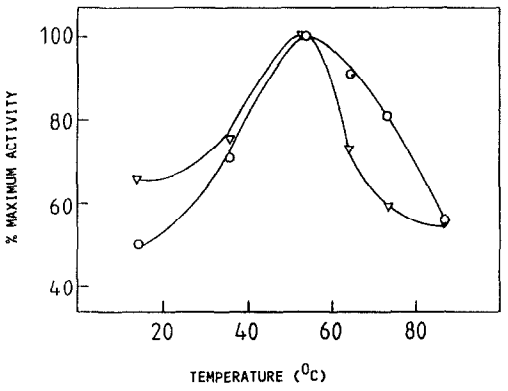

Figure 4 The effect of temperature on free and immobilized enzyme activity; poly(acrylamide/gelatin ratio: 2.5; ○ free urease; ▽ 0.08M chromium acetate cross linked urease

In order to determine the effect of reuse number on activity , different concentrations of CA (0.05M and 0.08M) were used during the immobilization steps for 2 different poly(acrylamide)/gelatin ratios of 4.2 and 2.5 (Figure 5).

As seen in Figure 5 curve A, 48 and 58% relative activities were obtained for 0.05M and 0.08M CA crosslinked ureases for 4.2 support composition after 20 uses. The highest relative activities were achieved for a pAam/PG ratio of 2.5 with 0.08M CA, as 71%, after 20 uses (Figure 5 curve B). For the same support composition when crosslinker concentration was used as 0.05M, relative activity was determined as 52%.

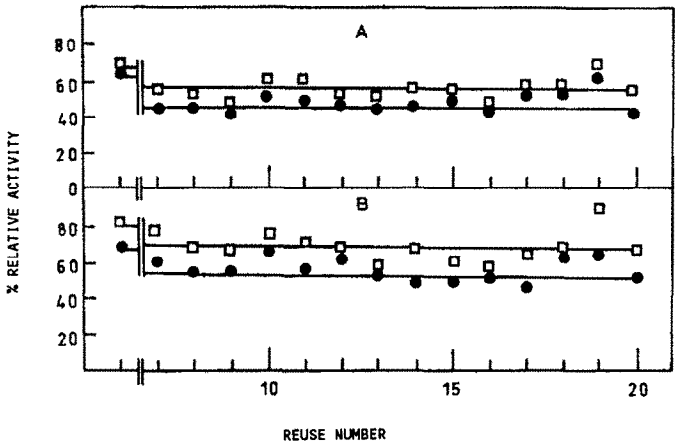

Figure 5 The effect of chromium acetate concentration and support ratio on relative activity of reused samples; poly(acrylamide)/gelatin ratios are 4.2 and 2.5 for A and B respectively; chromium acetate concentrations are ● 0.05M and □ 0.08M; free urease relative activity: 100

Conclusion- This study shows that CA crosslinked photographic gelatin and poly(acrylamide) can successfully be used for the immobilization of urease. Being an inexpensive inert material, PG is an advisable new support which gave good immobilization percentages when used together with a conventional support, pAam. When the results obtained in this work were compared with our previous study[8] addition of PG to pAam significantly increases the activity of immobilized urease.

The procedures applied during immobilization were cheap and easy to apply and as much as 71% of enzyme activity was retained after two months and 20 uses.This acceptable loss of activity can be attributed to structural changes in the enzyme during immobilization and possible decrease of reaction rate due to increased concentration of product within the pores. The stability of urease at low pH values was improved by immobilization but thermal stability was not effected considerably.

EXPERIMENTALS

Materials- Urease was obtained from Merck (lyophilized;5U/mg). Poly(acrylamide) was supplied from Aldrich having an average molecular weight of about 5-6 million. Granule type of photographic gelatin was obtained from Croda Gelatin Co. Polyester

films were purchased from Du Pont and had a thickness of 0.1mm which were precoated with a thin layer of photographic gelatin.

The substrate and the chemicals used to prepare reagents, buffer solutions,and the crosslinkers were obtained from Merck which were in analytical grade.

Apparatus- A Spectronic 20 D Milton Roy model spectrophotometer was used to determine the absorbance of the blue dye, indophenol.

Immobilization of Urease- Before the immobilization step, stock solutions of photographic gelatin (7.5%) and poly(acrylamide) (3.0%) were prepared in phosphate/EDTA buffer (pH 6.5) with mild heating (32-35° C) and vortexing. According to the type of immobilization, different proportions of these supports were taken and mixed thoroughly for 5 min. After, 0.5 ml of urease solution (40 mg/ml) was added and stirred again at 32° C. Different crosslinkers (CA and CS) with changing concentrations (0.02M-0.08M) were added to the immobilization gel, with vigorous stirring. Then, portions of 0.1ml of the gel were transferred onto polyester film strips (1x20cm) according to our earlier studies[11,12]. Enzyme leakage tests were realized by washing the strips with phosphate-EDTA buffer.

Determination of Urease Activity-Native and immobilized enzyme activities were detected by Berthelot method[13].1.5ml of Phosphate/EDTA buffer (pH 6.5) was added into the test tubes and enzyme strips were put inside the solution. After waiting at 37° C for 2 minutes (preincubation), by the addition of 0.2ml of urea (0.3g/l),the enzymatic reaction started. The incubation continued for 15 minutes with good mixing. Phenol-sodiumnitroprusside (5ml) and NaOH-hypochlorite (5ml) solutions were added at the end of the time to stop incubation and start color reaction. The solutions were rested for 30 minutes at 37° C. The absorbances were read at 555nm against the blank, after cooling.

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