Characterization of Amyloglucosidase Bound on Chitin

A S Rani, M L M Das and S Satyanarayana*
Department of Chemistry, Osmania University, Hyderabad 500 007, India

Received: 01 September 1998

Amyloglucosidase (AMG) from Aspergillus niger is one of the most important industrial enzymes. In industrial conventional enzymatic reactions, a mixture of substrate and native enzyme is incubated and after each batch of reaction the product is recovered by denaturation of the enzyme which cannot be reused because active site is lost which is an uneconomical process. The technique of immobilization of enzymes has been developed to circumvent many difficulties associated with the use of soluble enzymes.

In present investigation, AMG was immobilized on chitin without the aid of any cross linking agent and with retention of catalytic properties which can be used repeatedly and continuously. Chitin acts as a good supporting material for the immobilization of amyloglucosidase which is a homopolymer of N-acetyl-D-glucosamine. Immobilization of AMG on chitin was carried out by covalent binding method with the retention of 98 per cent of the catalytic activity of native enzyme. Amyloglucosidase can also be immobilized on ionotropic gel of chitosan by coupling with glutaraldehyde. Glucoamylase and α-amylase from the protease and glucosidase-less mutant HF-15 of Aspergillus awamori var. kawachi were immobilized on chitin without the aid of any cross-linking agents. To increase the efficiency of amyloglucosidase in industrial production of glucose, it has been customary to add another enzyme α-amylase to it. This made us to select dextrin as substrate rather than starch. In this investigation dextrin was prepared by adding α-amylase in the form of Sanzyme to gelatinized corn starch to increase the number of reducing ends by its random action on starch to enhance the rate of reaction of immobilized and native AMG. As conformational changes of enzyme protein may occur on immobilization and the affinity between enzyme and substrate may change, the investigation of optimum pH, optimum temperature, and kinetic constants of immobilized enzyme is very important.

Materials and Methods

AMG and Sanzyme were obtained from Novo-Nordisk Biomedical Group, Bangalore and Uni-Sankyo Ltd, Hyderabad, respectively. Locally available corn starch was used as a source of carbohydrate polymer. Powdered chitin from crab shells was purchased from Sigma Chemical Company, USA. Reducing values were determined by 3,5-dinitrosalicylic acid method and protein content in native and immobilized amyloglucosidases was estimated by Folin-phenol method.

Activities of AMG and Sanzyme were calculated as 3428 units and 26 units respectively. One unit is defined as 1 μ mole of glucose produced/min/ml. Protein content present in 1 ml AMG was estimated as 21.25 mg and in 1 ml of sanzyme as 20 mg. In our previous paper we have reported that AMG was immobilized on chitin by covalent binding method with the retention of 98 per cent of the catalytic activity of native enzyme. Activity of chitin immobilized AMG on dextrin hydrolysis was 3365 units (Figure 1). The activity of prepared immobilized AMG was 36.71 units/g of immobilized system. Protein content of the AMG present in 1 g of chitin was 0.2275 mg.

Assay of AMG — 1 g of corn starch was gradually suspended in 200 ml of 0.02 M acetate buffer at pH 4.2 and slowly heated on a water bath. Sanzyme (α-amylase complex) was added at the rate of 60 mg/g of starch. After liquification the resulting substrate was kept overnight at 4°C and used as a dextrin.
The activity of free as well as immobilized AMG was determined by 3,5-dinitrosalicylic acid method, using dextrin as a substrate in 0.02 M acetate buffer at pH 4.2 and incubating the mixture at 30 °C for 60 min. The liberated glucose was measured spectrophotometrically at 540 nm by DNS method for the activity of AMG.

Results and Discussion

The AMG of Aspergillus niger was tightly bound on chitin without the aid of any cross-linking agent. AMG contains specific binding site for chitin separate from the raw starch affinity site and active site, resulting into covalent binding.

Effect of pH — As enzymes are nothing but proteins, their catalytic activity is affected by environmental conditions, especially the pH of aqueous medium. Optimum pH of immobilized enzyme was 4.2 and the native enzyme optimum pH was 5 (Figure 2). Optimum pH of immobilized enzyme is shifted to acidic side by 0.8 units. This decrease is due to charge of the enzyme protein and/or of the water-insoluble carrier and also attributed to the microenvironment of immobilized enzyme which becomes more alkaline than external solution.

Effect of Temperature — Optimum temperature of chitin immobilized amyloglucosidase was 60 °C which decreased by 10 °C when compared to optimum pH of native enzyme 70 °C (Figure 3). This immobilized enzyme shows maximum activity at lower temperature (than native) which decreases the cost of production of glucose.

Kinetic Constants — Kinetic constants measured with immobilized enzymes are not true kinetic constants equivalent to those obtained in homogenous reactions, but are apparent values because of the effect of diffusion and other physical factors. So they are referred as apparent $V_{\text{max}}$ ($V_{\text{max(app)}}$) and apparent $K_m$ ($K_{m(app)}$). These are calculated by plotting simple graph by taking rates of enzyme hydrolysis reactions in mg against the concentration of substrate (dextrin) in mg.

$K_m$ value of chitin immobilized AMG is more than that of $K_m$ value of native enzyme (Table 1) which indicates that the stability of [E-S] complex is low and affinity between enzyme and substrate is less than that of native enzyme. Though the activity of chitin immobilized enzyme has 98 per cent of native enzyme activity, this is not as low as expected. So the high $K_{m(app)}$ of immobilized enzyme is due to diffusional limitations imposed on substrate in the presence of carrier. $V_{\text{max(app)}}$ of immobilized enzyme was less than that of native enzyme value which is in agreement with the activity of the enzyme. This immobilized system

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$K_m$ (g/l)</th>
<th>$V_{\text{max}}$ (g/min/unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free AMG</td>
<td>$1.12 \times 10^{-3}$</td>
<td>$4.49 \times 10^{-4}$</td>
</tr>
<tr>
<td>Immobilized AMG</td>
<td>$1.16 \times 10^{-3}$</td>
<td>$4.28 \times 10^{-4}$</td>
</tr>
</tbody>
</table>
has exciting opportunities in the developing energy-efficient and cost-effective processes (Rani A S, personal communication).

Continuous Production of Glucose in Laboratory Scale
— A column of 2.4 cm in diam and 57 cm in length was loaded with the Chitin-immobilized AMG to give a total bed volume of 240 mL. The column was equilibrated with 0.02 M acetate buffer (pH 4). 10 per cent dextrin solution was fed continuously in the column with inflow and outflow rate of 12 ml/h. A small amount of toluene was periodically added to the substrate solution to prevent microbial contamination. Completion of hydrolysis of dextrin was detected by a spot test and the amount of glucose produced was estimated by DNS method. Glucose was the only product obtained in the solution in good yields i.e., 420 mg/5ml. This column was stable for 25 d without losing the activity.

Conclusions
Chitin immobilized AMG shows 98 per cent of the native enzyme activity which is very high when compared to AMG immobilized on other carriers (Rani A S, personal communication). This immobilized system can be employed in the industrial production of glucose from dextrin which can be used repeatedly and continuously. Chitin is a good supporting material for immobilization and drying leads to decrease in activity. Production of glucose was maximum at pH 4.2 and 60 °C. This column using immobilized enzyme can be recycled and reused after long duration.

Acknowledgement
The authors are grateful to CSIR, New Delhi for awarding SRF to A S Rani and M L M Das.

References