Properties of Glucoamylase Immobilized on Soluble Polymer

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Glucoamylase (α -1, 4 Glucan glucohydrolase, Exo-1, 4, α -glucosidase E. C. 3.2.1.3.) from *Rhizopus niveus* was immobilized to cyanogen bromide-activated dextran. The glucoamylase-dextran conjugate was purified by gel filtration chromatography. It was found that 92% of the original protein of the enzyme was recovered in the conjugate and about 82% of the specific activity of the native enzyme retained. Some properties of the enzyme conjugate were investigated and compared with those of the free enzyme. The pH activity and pH stability of the immobilized enzyme were not much altered with the optimum pH at 5.5 as that of the free enzyme. Immobilized enzyme exhibited a wide range of temperature stability of 50~60°C as compared to the free enzyme. Heat inactivation at 55°C had less effect on the residual activity of the immobilized enzyme in which 60% activity was retained after prolonged heating for 60 minutes.

Immobilization of amylase and glucoamylases on organic and inorganic supports has been investigated by several workers¹⁾⁻⁶⁾. Glucoamylase was immobilized on various water-insoluble supports such as DEAE-cellulose¹⁾, granular polyacrylonitrile (PAN)²⁾ and silanized alkylamine glass beads (CPG)³⁾. However, some of the more important industrial substrates such as starch and cellulose will not be effectively acted upon by insoluble forms of immobilized enzymes due to the restricted access of enzyme to substrate. Recent works have shown that a few enzymes can also be attached satisfactorily to soluble supports particularly dextrans of varying molecular weight⁷⁾⁻¹⁰⁾, and high activity and high efficiency of coupling of the enzymes conjugated to cyanogen bromide-

activated dextran were also reported⁸⁾⁹⁾. However, little work has been reported on the attachment of glucoamylase to soluble polymers.

In the present study, the synthesis of glucoamylase-dextran conjugate and some properties of the conjugated enzyme are described.

1. Materials and Methods

Materials

Glucoamylase (E. C. 3.2.1.3., α -1, 4 glucan glucohydrolase, Exo-1, 4, α -glucosidase) originating from *Rhizopus niveus* was purchased from Seikagaku Kogyo Co. Ltd. The enzyme was in a lyophilized form with the activity of 30 Units/mg. Dextran (average m.w. 170 000 ~200 000) was from Nakarai Chemicals Ltd. Cyanogen bromide, extra pure grade, was obtained from Kanto Chemical Co. Cross-linked dextran in the form of Sephadex G-200 (particle size 40~120 μ m) was from Pharmacia Fine Chemicals. All other Chemicals were of reagent grade.

Methods

(1) Preparation of glucoamylase-dextran conjugate

Glucoamylase was covalently bound to soluble dextran using the cyanogen bromide activation technique as described by Kuo and GOLDSTEIN⁹⁾. The enzyme-dextran conjugate was separated from unreacted enzyme by Sephadex G-200 gel filtration chromatography at 4°C by using 50 mM phosphate buffer as eluent.

(2) Glucoamylase assay

The activity of free and immobilized glucoamylases was assayed at 40°C, 15 min by using 1% soluble starch in 0.05 M acetate buffer at pH 4.5 as substrate. The amount of glucose produced was determined by the NELSON-SOMOGYI method¹²⁾¹³⁾. Protein content of enzyme solutions was determined by the method of LOWRY¹¹⁾.

One unit of glucoamylase activity was the amount of enzyme which liberated 1 μ mole of glucose per min under the above conditions.

2. Results and Discussion

Extent of conjugation

Absorbance measurement at 280 nm of the

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eluate fractions revealed the presence of 3 elution regions. The first region (dextran- α -amylase conjugate) showed high enzyme activity whereas the second (free α -amylase) possessed very little activity. No activity was observed in the third region. It was found that 92% of the starting protein was retained in the enzyme conjugate. Specific activity as expressed in terms of glucoamylase unit per mg of protein was 27.72 units which represents 81.8% of that of the native enzyme (33.88 units) when both were assayed at pH 4.5 in 0.05 M acetate buffer.

pH profile of immobilized enzyme

The pH activity curves for both enzymes were closely similar with maximum activity at pH 5.5. However, the immobilized enzyme also exhibited the optimum pH activity at pH 6.0 (99% relative activity).

The pH-activity profile of immobilized glucoamylase was shifted by 0.2~0.5 pH units to the alkaline side. The free enzyme showed slightly higher activity in the acidic pH regions between 2.5 and 4.5. The stability towards various pH buffers of immobilized enzyme was not different from that of the free enzyme.

Temperature stability of immobilized enzyme

The optimum temperature for enzyme activity was determined by incubating the enzyme solution at various temperatures between 37 to 40°C in the presence of 1% soluble starch in 0.05 M acetate buffer (pH 4.5). The immobilized enzyme exhibited a wider range of maximum activity (50~60°C) than the free enzyme (50°C). Both forms of enzyme lost about 80~85% of their activities at 65°C and about 90% at 70°C (Fig. 1).

Free and immobilized enzyme solutions, containing about 3.5 μ g protein in 0.05 M acetate buffer (pH 5.5) were preincubated for 10 minutes at different temperatures between 37 and 60°C in the absence of substrate. The immobilized enzyme was more resistant to heat inactivation than the free enzyme during the temperatures 50~55°C. The loss of activity was only 1% at 50°C and 12% at 55°C, while the free enzyme lost 13 and 20% of its activity at 50 and 55°C, respectively.

The enzyme still retained 60% of its activity

Fig. 1 Thermal stability of free (—○—) and immobilized (—●—) glucoamylase based on 15 min reaction at different temperatures in 50 mM acetate buffer at pH 4.5

Fig. 2 Heat inactivation of free (—○—) and immobilized (—●—) glucoamylase

Enzyme solution were held at 55°C in 50 mM acetate buffer at pH 4.5 and samples were removed at 10 min intervals for the assay.

after heating for 60 min. Free enzyme was more unstable than the immobilized enzyme with the loss of 10 and 60% activity after 20 and 60 minutes, respectively (Fig. 2).

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可溶性高分子に固定化したグルコアミラーゼの性質

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グルコアミラーゼ (*Rhizopus niveus* 起源 α -1, 4 グルカングルカノヒドロラーゼ) の水溶性高分子への固定化法とその特性について検討し。グルコアミラーゼは、シアノゲンブロマイドで活性化したデキストランへ固定した。ついで、セファデックス G-200 カラムにより未反応の遊離酵素を除いて固定化酵素を精製した。使用した酵素タンパク質の 92% が固定され、活性の 82% が保持される結果を得た。固定化グルコアミラーゼの最適 pH, pH 安定性は、未処理の酵素とほぼ同じ値 (pH 5.5) を示した。しかしながら、本固定化酵素の特長は熱安定性に認められ、未処理酵素の 50°C に対し、50~60°C の広い範囲で安定であり、55°C, 60 分の加熱処理に対しても 60% の残存活性を示した。