Determination of Glucoamylase in Culture Filtrates Ccontaining Other Amylolytic Enzymes

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An improved method is proposed for the assay of glucomaylase in the presence of other amylolytic enzymes by using low molecular weight dextran as a more specific substrate. Activity is quantified by the amount of glucose produced as determined by a glucose oxidase-peroxidase-chromogen reaction.

Bestimmung der Glucoamylase in Kulturfiltraten, die andere amylolytische Enzyme enthalten. Es wird eine verbesserte Methode fur die bestimmung der Glucoamylase in Gegenwart anderer amylolytischer Enzyme vorgeschlagen. Sie beruht auf der Verwendung von Dextran mit niedrigem Molekulargewicht als spezifisches Substrat. Die Bestimmung der aktivitat erfolgt durch quantitative Ermittlung der Glucosemenge mit Hilfe einer Glucoseoxidase-Peroxidase-Chromogen-Reaktion.

Introduction

Glucoamylase (1,4-alpha-D-Glucan Glucohydrolase EC 3.2.1.3.) is one of the less specific amylolytic enzymes since it cleaves alpha-1,3-, alpha-1,6- and alpha 1,4- linkages [1,2]. Its activity is assayed in two steps; firstly hydrolysis of the substrate and secondly the the determination of the resulting glucose. The prefered method of glucose assay is enzymatic, using a glucose oxidase-peroxidase-chromogen system. In fungal cultures, glucoamylase rarely occurs without alpha-amylase [3]. Other amylolytic enzymes such as alpha-glucosidase are also likely to be concommitantly produced. This presents a difficulty in accurately comparing glucoamylase productivity between culture strains simply by assaying the crude culture filtrate. Glucoamylase activity in crude culture filtrates containing such mixtures of amylolytic enzymes when assayed using non-specific substrates such as starch (most frequently used), malto-oligosaccharides and maltose, is prone to overestimation due to other influences on the rate at which glucose is produced. These influences include generation of chain-ends by alpha-amylase and non-glucoamylase produced glucose e.g. by alpha-glucosidase. However, these enzymes cannot cleave alpha-1,6 linkages [4].The dextran produced by various species of Leuconostoc mesenteroides is a predominantly alpha-1,6 linked polysaccharide [5].In studies of dextran structure, glucoamylase was used successfully as an exodextranase on dextrans of various molecular weights [6]. Glucoamylase assays using an alpha-1,6 linked dextran as substrate will deny contribution to the rate of glucose production by amylolytic enzymes unable to cleave alpha-1,6 linkages. Dextran is therefore a more specific substrate for glucoamylase. This paper reports a method for assaying glucoamylase using dextran.

Materials and Methods

Reagents

Glucoamylase from Rhizopus sp. (Product number A7255; 2200 units g^{-1}; Sigma Chemicals, St. Louis, Missouri, U.S.A. ; Dextran T-10 (average molecular weight of 9700)(Pharmacia Fine Chemicals, Uppsala, Sweden) produced from Leuconostoc mesenteroides NRRL-B512, containing 95% alpha-1,6 linkages in main and side chains [7]; glucose oxidase-peroxidase diagnostic kit for the determination of glucose (Sigma Chemicals), all other chemicals were of analytical grade.

Enzyme Assay

Hydrolysis of Dextran T-10

Solutions containing 10 - 70 units (where a unit as defined by supplier, will liberate 1 mg of glucose from soluble starch in 3 min. at pH 4.5 at 55°C) of glucoamylase were prepared using 0.1M citric acid - 0.2M sodium dihydrogen phosphate (McIllvaine) buffer [8] made to pH 4.5 at 35°C. Insoluble material was centrifuged out and only the supernatant was used.Hydrolysis was performed by adding 0.5 mL

aliquots of enzyme solution to 2.5 mL of 1% Dextran T-10 dissolved in the above buffer. Incubation was for 1 hour at 35oC. The reaction was stopped by the addition of 3.0 mL of 6% (w/v) trichloroacetic acid. Blanks contained 2.5 mL of substrate solution only. At the end of incubation, 3.0 mL of 6% trichloroacetic acid was added, followed by 0.5 mL of the various glucoamylase solutions.

Determination of glucose

A 0.5 mL sample of hydrolysate or solution from each of the tubes above was added to 5.0 mL aliquots of glucose oxidase-peroxidase-chromogen solution. In the standard, 0.5 mL of a 1:14 dilution of a 1 mg mL-1 glucose standard solution (Sigma Chemicals) was added instead of hydrolysate. Incubation was for 30 minutes at 37°C. The optical density of the solutions were then determined at 450 nm in a Pye Unicam SP8-150 spectrophotometer using a 1 cm cuvette.

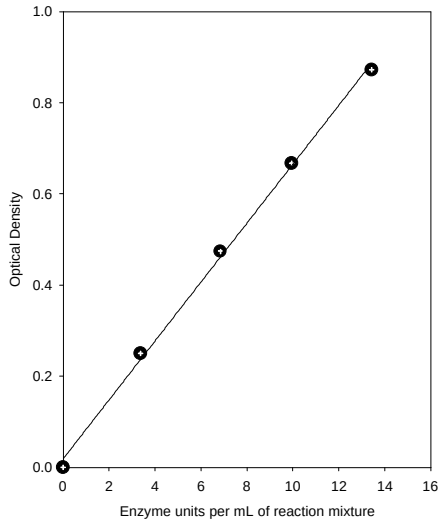

Fig. 1. Optical densities resulting from glucose oxidase-peroxidase estimations of glucose in Dextran T-10 hydrolysates produced using different glucoamylase concentrations; 0.5 optical density unit is equivalent to 0.11 mg ml^{-1} glucose in the hydrolysate.

Results and Discussion

A linear relationship exists between enzyme concentration and optical density in the range of 0 - 13 units (Figure 1). This more than covers the concentration that glucoamylase is likely to occur in culture filtrates.

This assay method provides for a relatively accurate determination of glucoamylase in crude culture filtrates produced by different culture strains without the need for purification. The substrate is easily dissolved to give a clear solution and the use of a strong buffer for hydrolysis means that pre-treatment of culture filtrate with respect to pH is not often required. This method is currently being used in a mutant screening programme to improve glucoamylase productivity. A limitation to this assay is that it loses its advantage in specificity if the culture produced dextranase (1,6-alpha-D-Glucan 6 - glucanohydrolase, EC 3.2.1.11).

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