CONVERSION OF CELLULOSE TO SUGARS AND CELLOBIONIC ACID BY THE EXTRACELLULAR ENZYME SYSTEM OF CHAETOMIUM CELLULOLYTICUM

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SUMMARY

Hydrolyses of cellulosics by crude enzyme yielded glucose, cellobiose, and cellobionic acid. A cellobiose oxidizing enzyme was detected in culture broth of Chaetomium cellulolyticum and identified as a cellobiose dehydrogenase.

INTRODUCTION

The ascomycete C. cellulolyticum produces on induction the complete cellulase complex, i.e. endoglucanase, exoglucanase, β-glucosidase, and hemicellulase (Fühnrich and Irrgang, 1981, 1982). In the course of our investigations on SCP production and cellulase formation, we also studied the feasibility of cell free enzymatic hydrolysis of cellulosics by the C. cellulolyticum enzyme system. The present study was conducted with unprocessed newspaper as a model for cellulosic waste substrate, and with the delignified amorphous cellulose Solka Floc. The kinetics of carbohydrate formation and the extent of cellulose degradation were studied under batch and membrane reactor conditions. In addition, it is briefly reported on the occurrence of an extracellular enzyme of C. cellulolyticum, which catalyzes the oxidation of cellobiose in the presence of suitable electron acceptors.

MATERIAL AND METHODS

Enzyme Source and Hydrolysis of Cellulose. Enzyme preparations were obtained from culture broths of C. cellulolyticum ATCC 32319 and its mutant 73/7 grown on newspaper as described earlier (Fühnrich and Irrgang, 1981). Culture filtrates were concentrated by ultrafiltration and dialyzed (Amicon PM-10 membrane, dialysis buffer 0.05 M citrate, pH 4.3). Batch hydrolyses were performed under continuous shaking at 50°C in 20 ml glass vials containing 100 mg substrate suspended in 5 ml buffered enzyme solution. The hydrolysates were cleared by centrifugation and tested for sugar content. Membrane reactor hydrolyses were carried out in a stirred ultrafiltration cell (Amicon model 202, PM-10 membrane). The initial amounts of substrates were 4 g. The reaction mixture in the cell was maintained at a volume of 200 ml, the effluent flow rate and the buffer feed were adjusted by a peristaltic pump at 53 ml/h. Effluent fractions of 7 to 14 ml were collected for carbohydrate assays.

Substrates. a) Shredded newspaper, containing 50% cellulose as determined by the anthrone reagent after Undegraft (1959).
Pretreatment procedure with NaOH was as reported earlier (Pähnrich and Irrgang, 1981). b) Solka Floc BW-100, provided by Brown Co., Berlin, N.H., USA.

Carbohydrate Determinations. Reducing sugar was determined according to Nelson (1944), individual carbohydrates by anion-exchange chromatography on an automated carbohydrate analyzer (ZA 5100, Biotronik, München, Germany). Samples were applied to a 6 x 550 mm column packed with Durrum DA-X4-20 resin in the borate form, and separation was achieved at 60°C by a two-step buffer elution with 0.4 M H₂BO₃ (pH 9.4) and 1 M H₂BO₃ (pH 9.4). Flow rate of buffer was 60 ml/h, and of reagent 70 ml/h (0.1% orcinol in conc. H₂SO₄). Fig.1 shows a typical chromatogram of a hydrolysate obtained in membrane reactor hydrolysis of newspaper.

Protein Separation by Anion-Exchange Chromatography. The fractionation was carried out on a 25 x 550 mm column packed with DEAE-Trisacryl M (J&W instrument, Grünfelding, Germany). Elution was performed at 4°C with 10 mM Tris-HCl buffer, pH 7.5, and a linear gradient of 0-1 M NaCl. The flow rate was 20 ml/h, and 10 ml fractions were collected.

Thin-Layer Chromatography (TLC). Separation and fluorimetric detection of "sugar acids" was carried out after the method of Ghibitz et al. (1976). Merck pre-coated TLC plates SIL G-25 were used, the mobile phase was isopropanol-ethyl acetate-water-conc. ammonia (35:20:25:25, v/v).

Enzyme Assays. Activities of endoglucanase, exoglucanase, aryl-β-glucosidase, celllobiase, and xylanase were determined as previously described (Pähnrich and Irrgang, 1981, 1982). Celllobiase dehydrogenase was assayed according to Ander and Eriksson (1977) with 3,5-di-tert-butyl-o-benzoquinone and celllobiose as substrates. The reduction of quinone was followed at 40°C, pH 4.5, and 420 nm on a Zeiss spectrophotometer PM 6. One unit was defined as the amount of enzyme which reduced 1 µmol/min of quinone (the experimentally determined molar absorption coefficient E₄₂₀ of 1.423 cm²/µmol was used).

RESULTS AND DISCUSSION

Enzymatic Hydrolysis of Newspaper. Hydrolysates were carried out with unbleached material and concentrated enzyme solution. The time course of batch hydrolysis showed a rapid initial increase of total sugar and glucose during the first 6 h of incubation (Fig.2). Both the reducing sugar and glucose continued to accumulate throughout incubation. Celllobiose accumulated for the first 2 h, thereafter its concentration fell. Xylose and xylobiose accumulated slowly, and represented only a fraction of 7% of the total sugars chromatographed after 24 h of hydrolysis. At 24 h glucose accounted for 81% of the total sugar, celllobiose for 9%, and the ratio of glucose-celllobiose was 9:1. When the incubation time was extended to 48 h, only a slight further increase in glucose could be observed. About 50% saccharification of the newspaper cellulose was achieved. The decline in saccharification observed can be attributed to end-product inhibition and heat instability of the Chaetomium cellulase system, as has been shown recently (Pähnrich and Irrgang, 1982). In order to reduce end-product inhibition, hydrolysates were carried out in a membrane reactor. The hydrolysate products were continuously removed, and to follow the changes in concentration of individual carbohydrates during hydrolysis,