INFLUENCE OF 2-DEOXY-D-GLUCOSE ON GALACTOFURANOSIDASE AND PEPTIDOPHOSPHOGALACTOMANNAN SYNTHESIS IN PENICILLIUM CHARLESII

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SUMMARY

2-Deoxy-D-glucose inhibits synthesis of the glyco-enzyme exo-β-D-galactofuranosidase and its secretion into the growth medium of Penicillium charlesii cultures. In contrast, the synthesis of peptidophosphogalactomannan, an extracellular glycopeptide (peptido-polysaccharide) occurs in nearly normal quantities in the presence of 2-deoxy-D-glucose. The peptidophosphogalactomannan's composition in cultures containing 2-deoxy-D-glucose was comparable to that obtained from cultures containing no added 2-deoxy-D-glucose. We conclude peptidophosphogalactomannan is not derived from mural or extracellular glycoprotein(s) whose synthesis is inhibited by 2-deoxy-D-glucose.

INTRODUCTION

We have shown that the extracellular peptidophosphogalactomannan produced by P. charlesii is composed of a polysaccharide (phosphogalactomannan), and mannosyl-containing oligosaccharides and mannosyl residues attached to seryl and threonyl residues of a polypeptide (1). It contains 30 aminoacyl residues (2). The phosphogalactomannan is comprised of a mannan back bone to which 8-10 polygalactofuranosyl chains are attached. Each chain contains a variable number (2-15) of 5-O-β-D-galactofuranosyl residues. In addition, approximately 10 phosphodiester residues and ethanolamine are attached to the mannan. Exo-β-D-galactofuranosidase, a glyco-enzyme is secreted by P. charlesii (3). This enzyme removes galactofuranosyl residues from peptidophosphogalactomannan. 2-Deoxy-D-glucose inhibits glyco-invertase (β-D-fructofuranoside fructohydrolase, E. C. 2.3.1.26) synthesis and the synthesis of other glyco-enzymes (4,5,6 7,8). Higher concentrations of 2-deoxy-D-glucose are required before cell wall

glucan synthesis is inhibited appreciably (7). 2-Deoxy-D-glucose is presumably converted to GDP-2-deoxy-D-glucose, an analogue of GDP-D-mannose, and it inhibits mannosyl transferase (6).

The experiments described in this paper were conducted to determine if 2-deoxy-D-glucose inhibits the synthesis of two mannosyl-containing extracellular substances, i) exo-β-D-galactofuranosidase, and ii) peptidophosphogalactomannan.

MATERIALS AND METHODS

a. Peptidophosphogalactomannan isolation and analysis. Penicillium charlesii was cultured at 20° on a gyratory shaker (Model 10, New Brunswick Scientific Corporation) at a setting of 8 for 12 days and the peptidophosphogalactomannan isolated as described previously (1). Total carbohydrate was determined by the phenol-sulfuric acid method (9). The molar ratio galactose:mannose was determined following hydrolysis at 100° for 3 hr in 2 N H_2SO_4. The hexoses were converted to their trimethyl silyl derivatives and the quantity of each hexose was determined on an 8 foot column (3/16 in. OD) packed with 3% SE-52 on 80-100 mesh Gas Chrom Q. The column was attached to a Hewlett Packard model 5750 dual column gas chromatograph equipped with a flame ionization detector. The occurrence of ethanolamine and N-terminal amino acids was determined as described previously (2). β-Elimination of saccharides from the polypeptide was carried out in 0.4 N NaOH as described previously (2). The extent of dehydroaminoacyl formation was estimated from the absorbance at 241 nm. Protein was determined by the procedure of Lowry et al (10). ^{14}C was determined in a Beckman LS-230 scintillation spectrometer.

b. Isolation and determination of exo-β-D-galactofuranosidase. Exo-β-D-galactofuranosidase was isolated by passing culture filtrates, previously dialyzed against pH 4, 67 mM acetate buffer, over a Sepharose 4B-peptidophosphogalactomannan column. The galactofuranosidase was eluted with pH 4, 67 mM acetate buffer containing 0.5 M NaCl (3). The glyco-enzyme is stable at pH 4 and 25° for several days, and it is stable at 4° or -20° at pH 4 for at least a year.

In the assay for galactofuranosidase, peptidophosphogalactomannan containing 65 μmoles of galactofuranosyl residues was incubated for varying intervals at 37° in 67 mM acetate buffer at pH 4 in a total reaction volume of 105 μl. The quantity of galactose released was determined with galactose oxidase catalyzed reaction (11).

RESULTS

2-Deoxy-D-glucose inhibits extracellular galactofuranosidase formation (Fig. 1). Eight mM 2-deoxy-D-glucose, added to 3-day cultures, decreases the

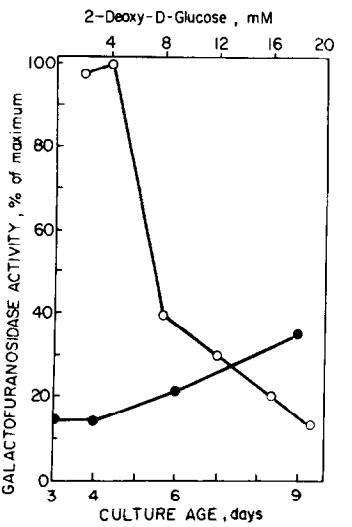

Fig. 1. Influence of 2-deoxy-D-glucose on the appearance of galactofuranosidase activity in Penicillium charlesii culture filtrates. Penicillium charlesii was cultured in a modified Raulin-Thom medium (13) as described in the text. Fifteen mM 2-deoxy-D-glucose was added to separate cultures 3, 4, 6, and 9 days after starting the culture. Twelve-day old cultures were filtered and the relative galactofuranosidase activity is given •—•. In a separate series of experiments 2-18 mM 2-deoxy-D-glucose was added to separate 3-day old cultures. The galactofuranosidase activity in 12-day old culture filtrates was determined and is shown, o—o.

galactofuranosidase activity in 12-day cultures to one-half that observed in cultures containing no 2-deoxy-D-glucose. 12-Day cultures, to which 15 mM 2-deoxy-D-glucose was added at 9 days, contained only 35% as much galactofuranosidase activity as a culture containing no 2-deoxy-D-glucose.

In contrast, 15 mM 2-deoxy-D-glucose added to 3-day cultures has negligible

affect on the mass of extracellular peptidophosphogalactomannan found in 12-day cultures. The composition of the polymer was examined and a molar ratio of galactose:mannose of 3:2 was found. 12-Day cultures containing no 2-deoxy-D-glucose have a galactose:mannose molar ratio of about 1:5, as compared to a ratio of 3:2 found in peptidophosphogalactomannan isolated from 5-6 day cultures which contain negligible quantities of galactofuranosidase activity. We proposed (3) that galactofuranosidase released into the growth medium at about 7-10 days caused the hydrolysis of galactofuranosyl residues in peptidophosphogalactomannan. The galactosyl residues in all preparations investigated were in the furanosyl configuration. These results provide additional evidence that P. charlesii cultured in 2-deoxy-D-glucose lacks appreciable galactofuranosidase activity in the culture filtrate.

Peptidophosphogalactomannan obtained from cultures containing added 2-deoxy-D-glucose was treated with 0.4 N NaOH to effect β-elimination of the saccharides. Mannose-containing saccharides were released similar to that reported for cultures containing no added 2-deoxy-D-glucose (1). The results of N-terminal amino acid analysis show the occurrence of N-terminal seryl, glycyl, aspartyl/asparginyl, and glutamyl/glutaminyl residues. Dansyl-ethanolamine was also found in the hydrolysate. A 1.3 mg sample of peptidophosphogalactomannan was treated with 6 N HCl for 24 hr and the amino acid composition determined. Table I compares the quantities of each of the amino acids in the peptidophosphogalactomannan obtained from cultures containing no added 2-deoxy-D-glucose to cultures containing 15 mM 2-deoxy-D-glucose. Both the quantity and composition of the peptidophosphogalactomannan obtained from cultures containing added 2-deoxy-D-glucose are similar to that obtained from cultures containing no added 2-deoxy-D-glucose.

In order to provide more direct evidence for de novo synthesis of peptidophosphogalactomannan in the presence of 2-deoxy-D-glucose, a 3-day P. charlesii culture was made 15 mM with 2-deoxy-D-glucose, and [1-^{14}C]-D-glucose (200 μCi) was added to the culture 2 days later. The culture was filtered on the 12th

Table I

AMINO ACID COMPOSITION OF GLYCOPEPTIDES FROM CULTURES CONTAINING 2-DEOXY-D-GLUCOSE OR NO 2-DEOXY-D-GLUCOSE

AMINO ACID	Amino Acid Residues Per Mole[a]	
	Additions to Raulin-Thom Media	
	None	2-Deoxy-D-glucose 15 mM
	(1)	(2)
Serine	7.19	7.65
Threonine	5.62	5.34
Alanine	3.87	4.11
Glycine	2.52	2.91
Valine	1.66	1.62
Proline	2.21	1.86
Aspartate-asparagine	1.01	1.30
Glutamate-glutamine	1.43	1.83
Histidine	0.77	0.36
Isoleucine	0.53	0.45
Leucine	0.53	0.51
Lysine	0.36	0.39
Arginine	trace	0.18
Tryptophan[b]	trace	trace
Phenylalanine	trace	0.15
Tyrosine	trace	0.15
Ethanolamine	0.6	2.06

a Based on molecular weights of 26,500 (column 1), and 65,000 (column 2) which have been shown to be estimates of the molecular weights of polymers containing 15% and 70% galactose, respectively (2).

b Determined by the spectrophotometric method of Goodwin and Morton (12).

day and peptidophosphogalactomannan isolated. A sample (5 mg) was passed through a Sephadex G-50 column (3x85 cm) and all of the ^{14}C eluted in the void volume (Fig. 2a). Five mg of polymer was treated with 0.4 N NaOH and the products fractionated on Sephadex G-50 (Fig. 2b). Radioactivity was distributed in fractions 25-80, and carbohydrate appeared in fractions representing high molecular weight polymers (fractions 25-35) and low molecular weight saccharides (fractions 60-75). In addition, ^{14}C was detected in amino acids following hydrolysis in 6 N HCl and chromatography. These data show that all components of the pep-

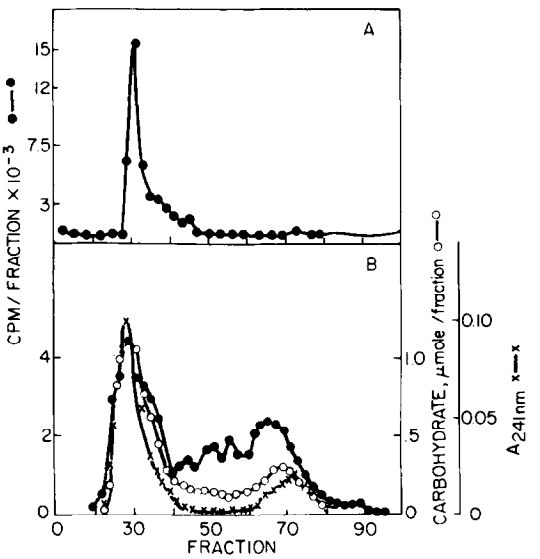

Fig. 2. Distribution of ^{14}C, carbohydrate and dehydroaminoacyl residues following β-elimination of saccharides from peptidophosphogalactomannan from P. charlesii. Penicillium charlesii was cultured in [1-^{14}C]-D-glucose as described in the text in the modified Raulin-Thom medium (13). Five mg of peptidophosphogalactomannan isolated from the culture filtrate was subjected to chromatography on a Sephadex G-50 (2.5x85 cm) column with 0.10 M NaCl as the eluent. The distribution of ^{14}C obtained in 5 ml fractions is shown in Fig. 2a. A second 5 mg sample of peptidophosphogalactomannan was treated with 0.4 N NaOH at 4° for 48 hr and after the sample was neutralized, it was fractionated on Sephadex G-50 (2.5x85 cm) column with 0.10 M NaCl as the eluent. The distribution of radioactivity, •—•, carbohydrate, o—o, and dehydroaminoacyl residues as measured by the absorbance at 241 nm, x—x, is shown in Fig 2b.

tidophosphogalactomannan are assembled by cultures which synthesize and secrete little exo-β-D-galactofuranosidase.

DISCUSSION

The synthesis and secretion of extracellular exo-β-D-galactofuranosidase is blocked by 2-deoxy-D-glucose, but the synthesis and secretion of extracellular peptidophosphogalactomannan is essentially unaffected by the presence of 2-deoxy-D-glucose in the culture. Further, 2-deoxy-D-glucose has no apparent effect on the composition of extracellular peptidophosphogalactomannan released by P. charlesii other than to provide a polymer that contains approximately 70% galactofuranosyl residues. Farkas et al. (7, 8) reported that a molar ratio of

glucose:2-deoxy-D-glucose of 20:1 inhibited mannoprotein synthesis in Saccharomyces cerevisiae, but that cell wall glucan synthesis was not inhibited by these conditions. Because both extracellular exo-β-D-galactofuranosidase and peptidophosphogalactomannan contain mannosyl residues we anticipated that 2-deoxy-D-glucose would effectively inhibit the synthesis of both substances. However, our experiments show that 2-deoxy-D-glucose does not block synthesis of all mannose-containing polymers. The results suggest that the mode of incorporation of mannose into peptidophosphogalactomannan must be different from that for glycoproteins.

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