STRUCTURAL-METABOLIC POLYSACCHARIDES OF Rhodotorula

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We have previously shown that Rhodotorula species produce intracellularly linear or weakly-branched mannans with an alternation of β-1→3 and β-1→4- bound mannopyranose units [1].

The soluble (structural-metabolic) polysaccharides of the cell walls of these yeast organisms are fairly complex in composition, and can be fractionated only with difficulty [2]. The present paper gives the results of a study of the structure of one of the fractions of the structural-metabolic polysaccharides of the cell walls of Rhodotorula species – glucomannans.

After testing different variants of the fractionation of polysaccharide preparations obtained by treating the yeast cells with dilute solutions of hydrochloric acid by means of Fehling's reagent, we isolated a fraction readily soluble in water and amounting to 14-30% of the weight of the initial materials which was characterized by a fairly constant composition (68-74% of mannose and 24-32% of glucose).

In the majority of cases, the formation of a copper complex took place not immediately but only 1-2 h after the addition of Fehling's reagent to an aqueous solution of the polysaccharide.

Attempts to eliminate the glucose component by repeated treatment with Fehling's reagent and by fractionation with dodecarbonium chloride under the conditions of the separation of mannans and glucans were unsuccessful.

On paper during electrophoresis in borate buffer, pH 9.3, experimental samples gave one band; on gel chromatography on Sephadex G-200 they gave one peak with $V_e = 36$ ml at $V_0 = 9.12$ ml. Chromatographic analysis of the fractions of the peaks showed the presence of mannose and glucose in the same amounts as in the samples subjected to gel chromatography. The characteristics of the glucomannans are given in Table 1.

Thus, the fractions isolated consisted of glucomannans of similar composition (ratio of glucose to mannose averaging 1 : 2.5). Table 2 gives the results of their periodate oxidation with subsequent tetrahydroborate reduction.

The results of a chromatographic study of hydrolyzates of the methylated glucomannans of Rh. lactosa and Rh. pallida (system 1, "Leningrad medium" paper, spots revealed with p-aniside hydrochloride) showed the presence of a spot with $R_g = 1$ at the level of tetramethyl derivatives, a very intense spot with $R_g = 0.82$-0.83 at the level of trimethyl derivatives, and a spot with $R_g = 0.59$-0.61 at the level of dimethyl derivatives.

TABLE 1

Glucomannan from	$[\alpha]_D^{+20}$, deg (0.25%, H_2O)	Reducing substances after hydrolysis, %	Amount, %			
			mannose	glucose	organic phosphorous	total nitrogen, %
Rh. glutinis	−83	94,0	68,0	24,0	0,05	0,30
Rh. rubra	−79	98,0	74,0	26,0	0,00	0,00
Rh. lactosa	−70	98,0	70,0	29,0	0,00	0,00
Rh. pallida	−76	99,0	69,0	29,0	0,10	0,00
Rh. marina	−75	97,0	70,0	28,0	0,00	0,00
Rh. minuta var. minuta	−83	96,0	68,0	27,0	0,00	0,00
Rh. minuta var. texensis	−70	97,0	68,0	32,0	0,00	0,00

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TABLE 2

Glucomannan from	Periodate oxidation					Products of tetrahydroborate reduction
	HCOOH	$NaJO_4$	nonreducing terminal groups or 1→6 bonds	1→4 and/or 1-2 bonds	1→3 bonds	
	mole/mole of hexose residue		%			
Rh. glutinis	0,18	0,73	18	37	45	Mannose, erythritol, glycerol, glucose (traces)
Rh. rubra	0,21	0,81	21	39	40	
Rh. lactosa	0,18	0,74	18	38	44	
Rh. pallida	0,18	0,70	18	34	48	

TABLE 3

Methyl glycoside	Amt. of methanolyzate, %	
	from Rh. lactosa	from Rh. pallida
Methyl 2,3,4,6-tetra-O-methyl-β-D-glucoside	2,0	1,7
Methyl 2,3,4,6-tetra-O-methyl-α-D-glucoside	12,3	12,1
Methyl 2,4,6-tri-O-methyl-β-D-glucoside	1,0	5,5
Methyl 2,4,6-tri-O-methyl-D-mannoside	37,8	39,4
Methyl 2,3,6-tri-O-methyl-D-mannoside	35,7	33,2
Methyl di-O-methylmannoside	11,3	8,0

When the fraction of the trimethyl derivatives was separated in system 2, two spots were found: an upper spot not giving a characteristic coloration with dimethylaniline and acquiring a reddish-brown tinge on subsequent treatment with p-anisidine hydrochloride, corresponding to a standard sample of 2,4,6-tri-O-methyl-D-mannose, and a lower spot giving a coloration with dimethyl aniline and acquiring a brown color after additional staining with p-aniside hydrochloride and corresponding to a standard sample of 2,3,6-tri-O-methyl-D-mannose. No other derivatives were revealed in the composition of the trimethylmannose fraction [1].

The spot of the dimethyl derivative was homogeneous, was not stained by dimethylaniline, and on demethylation (48% HBr, 30 min) it gave mannose.

The results of an investigation of the methanolyzates of methylated samples from Rh. lactosa and Rh. pallida by gas-liquid chromatography are given in Table 3.

Thus, it may be concluded (Tables 1-3) that one of the polysaccharides of the cell wall of the Rhodotorula species is a soluble glucomannan consisting of a branches β polymer. Its main chain is apparently a mannan similar to the extracellular mannans formed by the same species [1]. The side chains branch off from the mannose units in the C_2 or C_6 position, and many of them are possibly terminated by glucose units. A more detailed analysis of the side chains in the polymer will form the subject of a further investigation.

In a study of the IR spectra of the glucomannans, the same absorption bands were found as in the spectra of the extracellular mannans.

EXPERIMENTAL

The experiments were performed with Rh. glutinis 309, Rh. rubra BKM U341, Rh. lactosa 3-018, Rh. pallida No. 0715, Rh. marina No. 0928, Rh. minuta var. minuta BKM U338, and Rh. minuta var. texenis No. 0932.

The polysaccharide preparations were obtained from the cells of dry defatted yeast by treatment with 0.05-0.1 N HCl for 30 min in an autoclave at 0.5 atm [3]. The glucomannan fraction was isolated from the polysaccharide preparations by three treatments with Fehling's reagent [4] and was analyzed by methods described previously [5, 6].

Chromatography was performed on papers of types "FN-1" and "Leningradskaya srednaya" ["Leningrad medium"] in the following solvent systems: 1) butanol–ethanol–water–ammonia (40 : 10 : 49 : 1), and 2) methyl ethyl ketone saturated with water. The spots on the chromatograms were revealed with aniline hydrogen phthalate, an ammoniacal solution of silver nitrate, p-anisidine hydrochloride, and dimethylaniline.

The homogeneity of the fractions was checked by paper electrophoresis in a borate buffer, by gel chromatography on Sephadex G-200 [1], and by separating the glucomannan with dodecarbonium chloride under conditions specific for the separation of a mannan from a glucan [7].

The periodate oxidation of the samples with subsequent tetrahydroborate reduction was performed under conditions similar to those in the investigation of the extracellular mannans of Rhodotorula [7].

The glucomannans were methylated by Haworth's method (five times) [8] and then by the method of Falconer and Adams (twice) [9]. The products obtained did not show the presence of OH groups in the IR spectrum. The products of methylation were subjected to hydrolysis and methanolysis [1], and they were also studied by paper and gas-liquid chromatography ("Pye Argon Chromatograph", 3% of neopentyl glycol adipate on Chromosorb W, 60-80 mesh, 150 × 0.4 cm, 150°C, V = 30 ml/min, ionization detector).

CONCLUSIONS

1. One of the polysaccharides of the cell walls of the species of Rhodotorula studied in a structural-metabolic (soluble) glucomannan with a glucose : mannose ratio averaging 1 : 2.5.

2. The glucomannans of Rhodotorula species consist of branched β polymers, the main chain of which is a mannan consisting of 1 → 3- and 1 → 4-bound mannopyranose units and is similar in structure to the extracellular mannans formed by the same species.

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