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Preliminary communication

PRELIMINARY COMMUNICATION

A glucan from active dry baker's yeast (*Saccharomyces cerevisiae*): A chemical and enzymatic investigation of the structure

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Abstract: The structure of a polysaccharide consisting of D-glucose isolated from the cell-wall of active dry baker's yeast (*Saccharomyces cerevisiae*) was investigated by using methylation analysis, periodate oxidation, mass spectrometry, NMR spectroscopy, and enzymic hydrolysis, as a new approach in determination of structures. The main structural feature of the polysaccharide deduced on the basis of the obtained results is a linear chain of ($1 \rightarrow 3$)-linked β-D-glucopyranoses, a part of which is substituted through the positions O-6. The side units or groups are either a single D-glucopyranose or ($1 \rightarrow 3$)-β-oligoglucosides, linked to the main chaing through ($1 \rightarrow 6$)-glucosidic linkages. The low optical rotation as well as the ^{13}C-NMR and FTIR spectra suggest that the glycosidic linkages are in the β-D-configuration.

Keywords: active dry baker's yeast, *Saccharomyces cerevisiae*, polysaccharides, glucan, structure, chemical and enzymatic methods.

Considerable information on the structure of polysaccharides isolated from the cell-walls of baker's yeast (*Saccharomyces cerevisiae*) have already been reported.[1–3] The difficulties of obtaining and analysing purified wall components are numerous, because of the insolubility of some of them. When insoluble polysaccharides are associated with other cell wall polymers, the isolation becomes more difficult. The choice of suspending medium for the cell-wall isolation procedure also needs careful consideration. One of the most difficult problems in the purification of yeast glucans is their separation from close association with other insoluble polysaccharides, such as chitin and mannans. In recent years glucans from the cell-walls of many microorganisms have attracted a great deal of attention in view of their antitumor activity, especially the β-D-glucans.[4,5] Also, it was recently

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reported that a polysaccharide from the cell wall of brewer's yeast promotes the absorption of dietary iron and that the yeast glucan is the main polysaccharide having this effect.[6] In this communication preliminary results are presented of the isolation and structure determination of the glucan obtained from the cell-wall of baker's yeast (*Saccharomyces cerevisiae*), an important raw material for glucan production. Besides chemical methods, enzymatic hydrolysis, as a new approach in structure investigation, was also applied.

The active dry baker's yeast (commercial product made by Fermentation Industry "Alltech-Fermin", Senta, Serbia and Montenegro) as the polysaccharide material of the yeast cell-walls, was treated successively with acetate buffer (pH 5), 0.75 M sodium hydroxide, hot water, and 0.5 M acetic acid. The residue obtained by repeated extraction with acetic acid was dialysed and lyophilized. Further purification was achieved by salivary amylase and by pullulanase. The in this way obtained glycan was insoluble in water, alkalis, and most common organic solvents, but soluble in dimethyl sulphoxide, being one of the best solvents for many polysaccharides.

The pure polysaccharide gave one component after total acid hydrolysis, identified as D-glucose by paper chromatography and gas-liquid chromatography (GLC) analysis of the derived alditol acetates.[7]

The polysaccharide showed $[\alpha]_D^{20}$ –87º (c = 0.1 in DMSO) and displayed a band at 890 cm^{-1} in its FTIR spectrum, which is a characteristic feature of polysaccharides having the β-configuration. In addition there were bands at 2920, 1370, 1250, 1155, 1075, and 1040 cm^{-1} which indicate β-(1 → 3)-linkages.[8] The ^{1}H-NMR spectrum (DMSO-d_6, 200 MHz) of the polysaccharide contained signals at δ (ppm) 4.57 and 4.81 consistent with anomeric protons due to (1→6)- and (1→3)-β-D-glucosidic linkages, respectively.[9] In the ^{13}C-NMR spectrum (DMSO-d_6, 50 MHz) of the polysaccharide, signals were found at δ (ppm): 103.26; 86.47; 76.58; 73.05 and 68.68. Based on literature data,[10,11] these signals are assigned to carbon atoms of the 3-mono-O-substituted β-D-glucopyranosyl residue. The signal at 61.09 ppm corresponds to the C-6 of the non-reducing residue of the side groups and may also be assigned to 3,6-di-O-substituted units at branch point of the main chain, due to overlapping of the signals.

Additional evidence for the structure of this polysaccharide was provided by enzyme digestion by the (1→3)-β-D-glucanase of *Helix pomatia*. After incubation for 48 h, paper chromatography revealed two components, corresponding to glucotriose and gentiobiose.

The results of periodate oxidation[12] were in agreement with the above data. The polysaccharide consumed 1.30 mol of periodate and released 0.62 mol of formic acid per hexosyl residue. The Smith degradation of the polysaccharide,[13] *i.e.* borohydride reduction and hydrolysis of the periodate oxidized polysaccharide, afforded a mixture of two main components: glucose and glycerol, the first being the major component, as concluded on the basis of GLC of the alditol acetate derivatives (column DB-5, argon, 30 mL/min, FID).[7] The small amount of glycerol (≈ 10%) suggested either (1→6)-linkages or a slightly branched polymer of both. The D-glucose found in the hydrolysate of the periodate oxidized and reduced polysaccharide confirmed (1→3)-linkages and branching points.

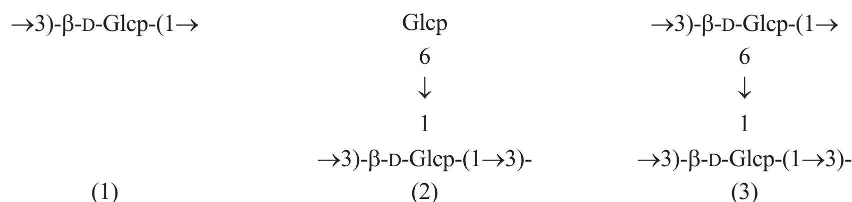
Methylation analysis substantiated the above results. The methylation was performed by the Hakomori procedure,[14,15] *i.e.*, by treatment of the polysaccharide with methyl iodide and methylsulfinyl carbanion in dimethyl sulfoxide. The per-*O*-methylated product (no hydroxyl absorption in the FTIR spectrum), $[\alpha]_D^{20}$ +5.8º (c = 0.15 in chloroform) on acid hydrolysis afforded a mixture of three components which by their chromatographic mobilities (see Table I) corresponded to 2,3,4,6-tetra-*O*-methyl-glucose, 2,4,6-tri-*O*-methyl-glucose, and 2,4-di-*O*-methyl glucose. Part of the hydrolysate was reduced, acetylated, and analyzed by GLC and GLC-MS (capillary column Supelco PTE-5, mass spectrometer "Finnigan Mat" model 8230).[16] The components were identified by their retention times and typical MS breakdown patterns obtained on electron impact. A summary of the results obtained by methylation analysis is shown in Table I.

TABLE I. Summary of the results of the methylation analysis of active dry baker's yeast (*Saccharomyces cerevisiae*) glucan

Cleavage product	Paper chromatography		Relative mole ratio[c]	Fragment ions (*m*/*z*)	Mode of linkage
	R_{Glc}[a]	R_{Glc}[b]			
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.00	0.78	1	45; 89; 117; 161; 205	Glcp-(1→
2,4,6-Tri-*O*-methyl-D-glucose	0.76	0.48	8	45; 117; 161; 201; 233	→3)-Glcp-(1→
2,4-Di-*O*-methyl-D-glucose	n.i.[d]	0.20	1	43; 87; 117; 129; 189; 233	→3, →6)-Glcp-(1→

[a] Butan-1-ol:ethanol:water = 4:1:5 (v/v); [b] Butan-2-one, saturated with water; [c] Based on the peak area by GLC; [d] Not identified.

The presence of 2,4,6-tri-*O*-methyl-glucose as the major component proves that D-glucose in the pyranoid form is incorporated into the main chain by (1→3)-linkages, a known linkage mode in many glucans from microorganisms and plants. The 2,4-di-*O*-methyl sugar, identified on the basis of combined evidence obtained by paper chromatography and GLC, as well as by GLC-MS analysis, indicates branching points, as represented by structures (1)-(3). 2,3,4,6-Tetra-*O*-methyl-D-glucose arises from nonreducing end units. The presence of small quantities of 2,4-di-*O*-methyl, and 2,3,4,6, tetra-*O*-methyl-D-glucose indicates that this glucan is slightly branched.



On the basis of these first results, a structure having a main chain composed principally of (1→3)-linked D-glucopyranoses (structure 1) may be proposed for the

homopolysaccharide. The main chain is 6-*O*-substituted with single D-glucopyranoses (structure 2) or (1→3)- β-oligoglucosides linked to the main chain through (1→6)-glucosidic linkages (structure 3).

Concerning the relationship between the structure of the investigated glucan of *Saccharomyces cerevisiae* with those of other yeasts, it is evident that the linear part of this polymer has a structure known in many yeasts glucans. A difference exists in the mode of branching, the content of side groups or chains, and in the configuration of the glycosidic linkages in the basic chain (α-D- and β-D-types).

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ИЗВОД

ГЛУКАН ИЗ СУВОГ АКТИВНОГ ПЕКАРСКОГ КВАСЦА (*SACCHAROMYCES CEREVISIAE*): ХЕМИЈСКО И ЕНЗИМСКО ИЗУЧАВАЊЕ СТРУКТУРЕ

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Испитивана је структура хомополисахарида изолованог из сувог активног пекарског квасца (*Saccharomyces cerevisiae*), методом метиловања, перјодатном оксидацијом, масеном спектрометријом, NMR спектроскопијом и ензимском хидролизом, као новим приступом у изучавању структуре полисахарида. Добијени резултати указују да полисахарид, који се састоји из D-глукозе, има (1→3)-D-глукополимерни низ као основни, а да су D-глукопиранозни остаци везани за основни низ преко положаја О-6 и то или као појединачне бочне јединице или као део олигомерних бочних група које чине (1→3)-D-глукопиранозил остаци. Конфигурација гликозидних веза је β-D-типа.

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