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(1→3)-β-D-GLUCAN FROM *SACCHAROMYCES CEREVISIAE*, PREPARATION, CHARACTERIZATION AND CHEMICAL MODIFICATION – INTRODUCING CARBONYL GROUPS INTO THE POLYSACCHARIDE CHAIN

Djordje ZEKOVIĆ[1*], Milanka RADULOVIĆ[2], Aleksanda NASTASOVIĆ[2], Miroslav M. VRVIĆ[2,3] and Dragica JAKOVLJEVIĆ[2]

[1]"Alltech-Fermin", Senta

[2]Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade

[3]Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

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A number of polysaccharides with β-glycosidic linkage are widespread in a variety of sources. All have a common structure, the (1→3)-β-D-glucan backbone is essential. They have attracted attention because of bioactive and medicinal properties which have been described for more than 50 years. Glucan from technologically important species, baker's yeast *Saccharomyces cerevisiae*, was isolated in a purified form and characterized. Data from common analytical methods supported by GLC-MS and NMR spectra indicated that isolated glucan from *Saccharomyces cerevisiae* has a backbone chain up of (1→3)-β-D-linked glucopyranoses with single β-D-glucopyranosul units at the branch points on every eight main chain unit.

* *Corresponding author*: "Alltech-Fermin", 24400 Senta, Karadjordjeva bb, Serbia. E-mail: zekadj@EUnet.yu

The oxidative transformation of β-D-glucan was investigated with dimethyl sulfoxide-acetic-anhydride reagent. Keto-aldehydo polymers were generated by treatment of β-D-glucan with this reagent. Accompanyng the oxidation was the formation of (methylthio)methyl groups along the polysaccharide chain at a degree of substitution smaller than the degree of substitution for the carbonyl group. As these groups groups are acid-labile, it has been found that can be removed by acid hydrolysis under conditions that not affect on the carbonyl content.

Key words: glucan, *Saccharomyces cerevisiae*, characterization, oxidation, dimethyl sulfoxide-acetic anhydride reagent

INTRODUCTION

Glucans with a backbone chain composed of (1→3)-linked D-glucopyranoses are widely distributed polysaccharides in microbes, especially in fungi. The fungal and yeast glucans have a common structure that comprises main chains of (1→3)-linked β-D-glucopyranosyl units along which are randomly dispersed side chains of β-D-glucopyranosyl units attached by (1→6) linkages (KOPECKA *et al.* 1974; Manners and others 1973). There is increasing interest in yeast β-glucans (SUTHERLAND IW 1998).

A number of (1→3)-linked polyglucoses is distributed in many bacteria, fungi, mushrooms, algae and higher plants and has attracted attention because of positive effects on human health; they have bioactive and medicinal properties, such as immune-stimulating, anti-inflamatory, antimicrobial, antitumoral hepatoprotective, cholesterol-lowering as well as antifibrotic, antidiabetic and hypoglycemic activity (ROBBINS and others 1977; SUTHERLAND 1998; BROWN *et al.* 2003; TREPEL 2004).

One important source of (1→3)-β-D-glucans is the cell wall of yeasts, particularly of the baker's and brewer's yeast *Saccharomyces cerevisiae* (STONE and CLARKE 1992).

In recent years, there has been an increasing interest in the preparation of macromolecular compounds which could have medicinal applications with polysaccharides as carrier molecules (MOLTENI 1979). Several polysaccharides has been used in this way, including dextran, starch and cellulose derivatives (YALPANI 1988).

In this paper we are exploring the possibilities of using β-D-glucans isolated from the cell walls of *Saccharomyces cerevisiae* in the preparation of chemically altered β-D-glucan with new reactive sites along the chain. This product possesses the same physical properties as β-D-glucan, but is able to undergo a much broader range of chemical reactions. In this paper, some promising results are presented on the application of mixtures of dimethyl

sulfoxide with acetic anhydride in order to obtain suitable reactive derivative useful for covalent coupling with potentially biologically active compounds. The utility of dimethyl sulfoxide-acetic anhydride mixture (DMSO-Ac_2O) as a convenient reagent for converting secondary and primary alcohol groups into keto and aldehyde groups (Albright and others 1965; Sweat and others 1967) has already been established in the carbohydrate field (HANESSIAN *et al.*, 1971; KATO *et al.*, 1990) but there are no data on the use of this reagent for the oxidation of native (1→3)-β-D-glucan.

In this paper, observations on the reaction oxidation of β-D-glucan by reagent dimethyl sulfoxide-acetic anhydride are presented. The aim of present work was to isolate (1→3)-β-glucans from the cell walls of *Saccharomyces cerevisiae* and prepare samples of oxidized glucan using a mixture DMSO-Ac_2O in order to broaden our knowledge about the products generated in this way, with new reactive sites along the chain and able to undergo a broader range of chemical reaction.

MATERIALS AND METHODS

General methods. Evaporations were conducted under reduced pressure at a bath temperature not exceeding 40°C. PC was performed on Whatman No.1 with A, ethyl-acetate-pyridine-water (2.5:1:2.5); B,1-buthanol-ethanol-water (4:1:5) C, butanone-water (azeotrope). GLC was performed at 170 °C on a Varian Model 1200 gas chromatograph column DB-5, argon, 30 mL/min, FID). GLC-MS was accomplished with an 8230 Finnigan Mat unit (capillary column Supelco PTE-5). The ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Varian Gemini 2000 instrument at 200 MHz (50 MHz). Elemental analyses (C, H, N and S) were performed at the Faculty of Chemistry, University of Belgrade.

Optical rotations were recorded at 20°C with a Perkin Elmer 141 MC polarimeter, and FT-IR spectra with a Perkin-Elmer Model 1725X spectrophotometer.

Isolation and purification of the (1→3)-β-D-glucan

The active dry baker's yeast was a commercial product produced by fermentation (sourced from "Alltech-Fermin", Senta, Serbia). The active dry yeast treated with 0.75 M sodium hydroxide at 60°C for 6 h. Distilled water was added to the dispersion. The insoluble part, after stirring for 1 h, was separated by centrifugation. The supernatant on acid hydrolysis yielded glucose, mannose and galactose. The precipitated material was suspended in 3% NaOH at room temperature for 3 h. The insoluble material was recovered by centrifugation (4000 rpm), washed three times with 60°C distilled water, centrifugated and resuspended in 0,5 M acetic acid at pH 4.5. This fraction on total acid hydrolysis afforded only

glucose. The residue, obtained by extraction with 0.5M acetic acid for 3 h at 90^{0}C, showed a slight positive stain with iodine and was subjected to seven such treatments with 0.5 M acetic acid, then dispersed in water and heated for 120 min at 121^{0}C. The insoluble residue was separated by centrifugation, suspended in distilled water and washed successively with ethanol, followed by ether and air dried. The glucan obtained in this manner was gradually dissolved in dimethylsulfoxide (DMSO) until total dissolution. Further purification was achieved using porcine amylase and pullulanase (details in next section).

The elemental analysis of the glucan (C 45.19%; H 6.38%, Ash 0.20%) showed satisfactory agreement with the calculated values for carbon and hydrogen.

Total hydrolysys

The polysaccharide (0.1g) was heated at 95^{0}C with 2 M trifluoroacetic acid for 16 h. The excess of acid was removed by repeated evaporations *in vacuo* and the resulting syrup was analysed by paper chromatography in systems A and B. An aliquot was reduced with $NaBH_4$, acetylated and analysed by GLC (SLONEKER 1972).

Enzymic digestions

The polysaccharide (0.15g) was suspended in 10 ml phosphate buffer (pH 6.9; 200 μmol)) and incubated with 4μkat α-amylase (Merck, EC 3.2.1.1) at 37 ^{0}C for 24 h. The inactivation of enzyme was performed by boiling for 5 min. After centrifugation, the supernatant was evaporated to a syrup and examined by paper chromatography in solvent A. Traces of oligosaccharides derived by amylolysis indicated the presence of small amounts of admixed glycogen. The residue after centrifugation was dialyzed against distilled water and freeze-dried.

The residue was subjected to the pullulanase enzyme (Merck, EC 3.2.1.41). The polysaccharide (0.1 g) was suspended in 10 mL of 0.03 M citrate-phosphate buffer (pH 5.0), containing 40 nkat of pullulanase and incubated for 24 h at 37 ^{0}C. After inactivation of enzyme by boiling for 10 min and centrifugation, the residue was dialyzed and freeze-dried. The supernatant was subject to paper chromatography in solvent A and no trace of oligosaccharide from admixed glycogen was detected.

The pure polysaccharide, having $[\alpha]_D^{20} = -8.7^0$ (c 0.15 in dimethyl sulfoxide), gave only one component after total acid hydrolysis. This was identified as D-glucose by paper chromatography (solvents A and B) and gas-liquid chromatography (GLC) analysis of the derived alditol acetates (SLONEKER 1972).

Methylation

Permethylation of glucan was performed according to the modified Hakomori procedure (HAKOMORI 1964).

To complete the process the product was methylated three times. The fully methylated product showed $[\alpha]_D^{20} = +5.8^0$ (c 0.15 in chloroform). The permethylated polysaccharide was hydrolysed (BOUVENG HO, 1965) with formic acid (90%) and sulphuric acid (0.25 M). The resulting partially methylated sugars were analysed by paper chromatography in solvents B and C, and then reduced with $NaBH_4$, acetylated, and analysed by gas liquid chromatography-mass spectrometry (GC-MS) (JANSSON 1976).

Methylation analysis of the glucan showed 2,3,4,6-tetra-O-methyl-glucose, 2,4,6-tri-O-methyl-glucose, and 2,4-di-O-methyl-glucose as the main methylated sugar derivatives in the relative molar ratio of 1:8:1, respectively.

Oxidation of the β-D-glucan

Samples of β-D-glucan (1 part) were suspended in a mixture of dimethyl sulfoxide (40 parts) and acetic anhydride (20 parts). The solutions were stirred for the specified time and at the specified temperatures, as shown in Table 1. The oxidized products were centrifugated (4000 rpm, 15 min) and the precipitated material washed with excess of water and the suspensions was dialysed against tap water, followed by distilled water. The resulting polymer was centrifugated and dried using ethanol and ether. The change in weight of the oxidized β-D-glucan was insignificant.

Determination of the carbonyl-group content

The carbonyl content was determined by the hydroxylamine hydrochloride reagent (VOGEL, 1966). This method was employed to convert the carbonyl (aldehyde and ketone) groups into corresponding oxime. The method consisted of treating a sample of oxidized β-D-glucan (0,05g) in the reagent (5ml) for 24h, and then washing it with ethanol. The resulting polyoxime was centrifuged and dried with ethanol and ether. The dry samples were analyzed by elemental analysis for nitrogen content. The percentage of nitrogen in polyoximes was calculated in related unmodified β-D-glucan which had been treated with hydroxylamine reagent in the same way. From the % N, the number of carbonyl groups per D-glucose residues was calculated. The percentage of sulfur in the oxidized samples of β-D-glucan was determined from results obtained from elemental analysis.

The selective conversion of the aldehyde groups into carboxyl groups

The selective conversion of the aldehyde groups into carboxyl groups was performed by oxidation with chlorous acid by a modification of the

procedure described by Ellington and Purves (ELLINGTON and PURVES 1953). The β-D-glucan (0.1g) which was oxidized at 25^{0}C for 2 days, was treated for 1 h at room temperature with 10 ml of 30% (v/v) aqueous acetic acid containing sodium chlorite (0,6 g). The acids were removed by centrifugation, the sample was washed with distilled water for 30 min and dialysed in distilled water for two days. The unmodified β-D-glucan was subjected the same procedure. Samples of glucans (each 0.02 g) were hydrolyzed with 2M trifluoroacetic acid at 95^{0}C for 18 h. Traces of acid were removed by repeated evaporations *in vacuo* and the resulting hydrolyzate was dried to a constant weight. The content of glucuronic acid was determined by the modified carbazol procedure (BITTER and MUIR 1962).

RESULTS AND DISCCUSION

The water insoluble glucan was isolated from the yeast biomass by consecutive alkaline/acid extraction and purification steps in the form of an aqueous suspension of swollen particles.

The pure β-D-glucan showed an optical rotation of $[\alpha]_D^{20} = -8.7$ (c 0.1, in dimethyl sulfoxide). This low optical rotation suggests the β-D-configuration of glycosidic linkages.

The FT-IR spectra of the glucan samples (Fig 1.) displayed absorption bands typical of a β-(1→ 3)-linkages. The band at 890 cm $^{-1}$ is a characteristic feature of polysaccharides having the β-configuration i.e. (C_1-H) deformation

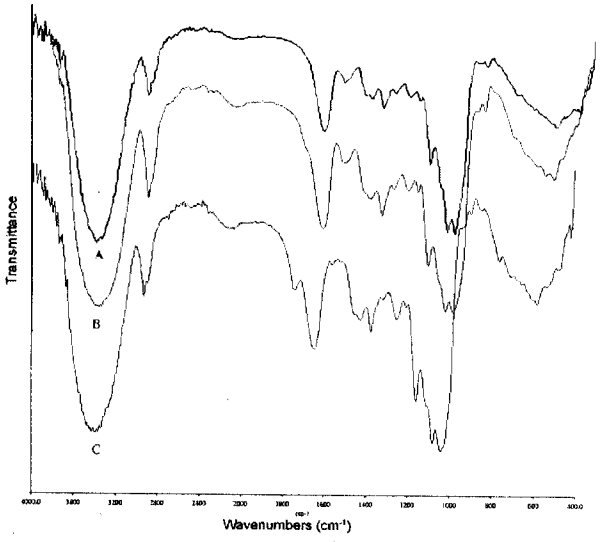

Fig.1. FT-IR spectra (in KBr) of the (1→3)-β-D-glucan before and after oxidation with dimethyl sulfoxide-acetic anhydride: A) purified (1→3)-β-D-glucan; B) oxidized (1→3)-β-D-glucan for 1 day; C) oxidized (1→3)-β-D-glucan for 3 days.

mode (Michell and others 1970) in addition to the bands that distinguish β-(1→3)-linkages at 2920, 1370, 1250 and 1200 cm^{-1} absorption bands arising from the v (CC) and the v (COC) stretching vibrations at 1160 cm^{-1} and two partially overlapping bands at 1078 and 1048cm^{-1} attributable to ring and (C-OH) side group stretching (MATHLOUTHI *et al.*, 1986). The FT-IR spectrum is shown in Fig. 1. (A).

Evidence supporting β-anomeric carbons in the β-glucan was confirmed by ^{13}C-NMR data. In the ^{13}C NMR spectrum (DMSO-d_6, 50 MHz) signals were found at (ppm): 103.26; 86.47; 76.58; 73.05 and 68.68. The ^{13}C NMR data did not reveal peaks corresponding to the α-configuration of the anomeric carbon; α-glucose anomeric carbons resonate at approx δ 100.00 ppm, and anomeric β-glucose carbons slightly downfield at δ104 ppm (SCHMID *et al.*, 2001).

The peak was only visible at δ 103.26 ppm strongly indicating that only β-anomeric carbons were present. The signal found at δ 61.09 ppm was attributed to the free C-6 from the principal region of the 3-O-substituted glucan. The 3-O-substituted carbon atoms from the main chain resonated at 86.5 ppm. The yeast glucan spectra were characterized by low signal intensities and significant line broadening, due to the formation of the gel network. This has been a general observation in ^{13}C-NMR spectra studies of high molecular weight glucans with β-(1→3)-D-linked backbone and arises from a tendency of these molecules to adopt an oredered conformation in solvent environments, which limits molecular motion of the carbons in the chain (SAITO *et al.*, 1977).

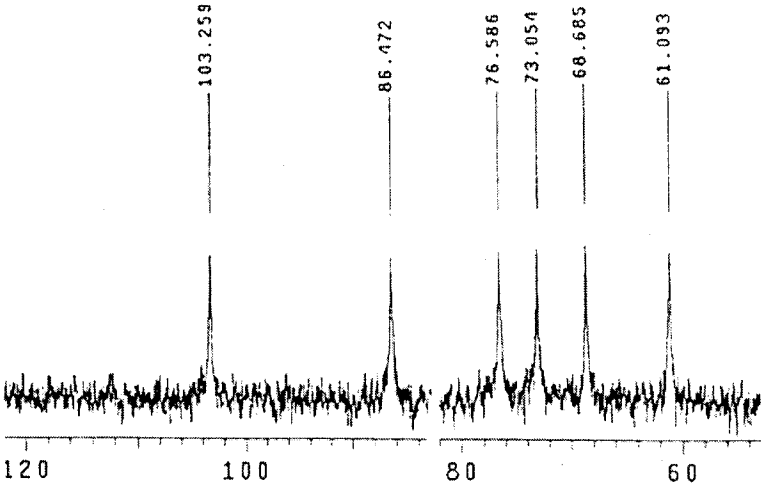

Fig. 2. ^{13}C-NMR spectrum of the purified (1→3)-β-D-glucan isolated from *Saccharomyces cerevisae*. Carbohydrate region shown.

The ^{1}HNMR spectra fractions of the β-glucan contained signals at δ 4.57 ppm attributable to 1,6-β-glucan moiety, and 4.81 ppm attributable to the non-reducing terminal, present at 3- and 6-substituted branching points (Kollar and others 1997; Usui and others 1975).

The elemental analysis of β-glucan show a satisfactory agreement with the calculated values for carbon and hydrogen. Results show that the polysaccharide was purified from proteins, nucleotides and chitin.

Methylation analysis of polysaccharide showed 2,3,4,6-tetra-O-methyl-glucose, 2,4,6-tri-O-methyl-glucose, and 2,4-di-O-methyl-glucose as the main methylated sugar derivatives in the relative molar ratio 1:8:1, respectively. This demonstrated that the polysaccharides was a (1→3)-β-D-glucan with a single (1→6)-β-D-glucopyranosyl side-branch attached along the (1→3)-linked glucosyl backbone chain. Thus, the tentative structure of the repeating unit of the yeast glucan can be described as backbone chain made up of eight (1→3)-linked β-D-glucopyranoses with a single (1→6)-β-D-glucopyranoses attached along the backbone chain, at a frequency of one branch point to every eight glucose residues as proposed SANDULA *et al.*, 1999.

Oxidation of β-D-glucan with DMSO-Ac_2O

The conversion of some hydroxyl groups of β-D-glucan to aldehyde and ketone groups in reaction with methyl sulfoxide-acetic anhydride mixture was investigated. Accompanyng the oxidation was the formation of O-(methylthio)methylpolysaccharide at a degree of substitution smaller than the degree of substitution for the carbonyl group.

Tab. 1. - Effect of reaction time on the oxidation and (methylthio)methylation of β-D-glucan

Temp. (°C)	Reaction time (days)	N_{oxime}[a] (%)	Carbonyl groups (miliequivalents per D-glucose residue)[b]	S[c] (%)	(Methylthio)methyl-groups (miliequivalents per D-glucose residue)[c]
25	1	0.38	43.97	0.61	30.88
25	3	0.58	67.11	0.54	27.33
25	5	0.71	82.16	0.49	24.80
25	8	1.14	131.91	0.51	25.82
25	12	1.18	136.54	0.43	21.76
5	1	0.29	33.55	0.23	11.64
40	1	1.23	142.32	1.18	59.73
60	1	1.39	160.84	2.05	103.78

[a] Calculated from % N determined by elemental analysis after complete conversion into the polyoxime

[b] Monomeric unit e.g. anhydroglucoside unit

[c] Calculated from % S found by elemental analysis

The oxidation of β-D-glucan was performed at room temperature and at 40°C and 60°C. In addition to the oxidation of β-D-glucan by this reagent, some (methylthio)methyl ether was also formed, as indicated by the sulfur content of the product. The total content of carbonyl groups (aldehyde + ketone) was reflected by the increase in nitrogen content, by elemental analysis, after reaction of the respective samples of oxidized β-D-glucan with the hydroxylamine reagent. The content of sulfur was determined by elemental analysis. The effect of reaction time and reaction temperature on the oxidation and (methylthio)methylation of β-D-glucan with this reagent is presented in Table 1.

FT-IR spectrum

The FT-IR spectrum, after treatment with the dimethyl sulfoxide-acetic anhydride reagent, clearly showed carbonyl absorption at ~1725 cm^{-1}. This was the only difference between the FT-IR spectra of β-D-glucan before and after oxidation with DMSO-Ac_2O mixture (Fig. 1) which indicated that some of the hydroxyl groups in the glucan had survived the oxidation. Further evidence that aldehyde or ketone groups had been generated along the β-D-glucan chain was the reaction of the treated β-D-glucan with hydroxylamine.

Table 1 shows the effect of varying the reaction times on the distribution of carbonyl groups (aldehyde and keto) in the oxidized product. The increase of this parameter led to a significant increase in carbonyl groups, and did not support formation of the (methylthio)methyl ether. The levels of oxidation and (methylthio)methyl ether formation are roughly similar at reaction times up ~1 day, after which the former reaction predominated.

Figure 3 shows dependence of the degree of substitution of nitrogen, assuming complete conversion of carbonyl groups into oximes, on the reaction time. The content of sulfur is also shown. As evident from Fig. 3, after a reaction time of 5 days, the degree of substitution of nitrogen increased significantly, while, on the other hand, the prolonged reaction time of oxidation led to a continual decrease of degree of substitution of sulfur. This was desirable, since (methylthio)methyl ether groups are byproducts of this reaction. The presence of (methylthio)methyl groups is a subsidiary reaction. However, it has been found that these groups can be removed under conditions that do not affect the carbonyl content. As these groups are base-stable, but quite acid-labile, a sample of β-D-glucan that had been treated with the dimethyl sulfoxide-acetic anhydride reagent, was boiled in a 3.3M acetic acid solution for 30 minutes to cleave (methylthio)methyl groups, thus giving the sulfur free product, with no loss in carbonyl groups.

The selective conversion of the aldehyde groups into carboxyl groups was performed by oxidation with chlorous acid (Ellington and Purves 1953).

This second oxidation step using acidified sodium chlorite solutions selective oxidizes aldehide groups and a final product contains carboxylic acid groups which content was estimated. Glucuronic acid was determined after total acid hydrolisis using the carbazole assay (BITTER and MUIR 1962).

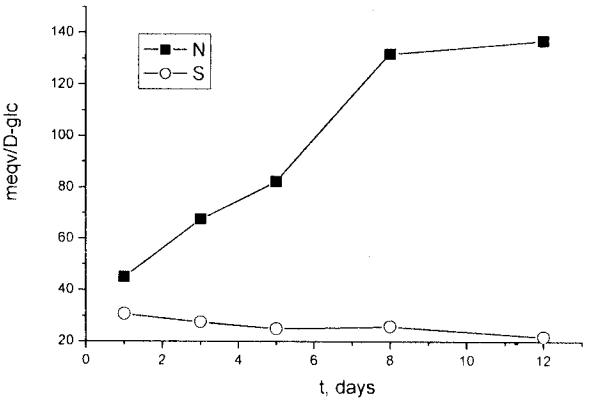

Fig. 3. Depedence of the (d.s) of carbonyl and (methylthio)methyl groups on the reaction time

Determination of the functional groups in oxidized β-D-glucan

The possibility of the production of three types of carbonyl group (aldehyde, keto and carboxylic) in β-D-glucan oxidized by the DMSO-Ac_2O was examined. From the data obtained from the further oxidation with chlorous acid, the number of carboxyl groups in the sample of oxidized β-D-glucan was assayed

Tab. 2. - Data of the perecentage of the aldehyde groups in β-D-glucan oxidized with dimethyl sulfoxide-acetic anhydride

β-D-glucan	COOH groups (meqv/D-Glc residue)	Carbonyl groups (meqv/D-Glc residue)[a]	Aldehyde groups (% of total carbonyl groups)[b]
β-D-glucan treated with chlorous acid	1	0	/
β-D-glucan oxidized[c] with mixt. DMSO/Ac_2O	2	31	/
Oxidized β-D-glucan treated with chlorous acid	21	/	61.3
β-D-glucan oxidized[c] with mixt. DMSO /Ac_2O	2	45	/
Oxidized β-D-glucan treated with chlorous acid	24	/	48.8

[a]From oxime oxidized β-D-glucan with dimethul sulfoxide-acetic anhydride
[b]Determined spectrophotometrically by carbazole assay
[c]Oxidation of unmodified β-D-glucan with mixt. DMSO/Ac_2O at 25°C for 2 days

with the carbazole assay after total hydrolysis. The difference in carboxyl content of the oxidized β-D-glucan, before and after further oxidation with chlorous acid, was related to the content of the aldehyde group present in the native sample. As the total content of carbonyl groups could be determined by the hydroxylamine hydrochloride reagent, from this data the content of aldehyde groups can be calculated. This is illustrated in Table 2.

Hydroxylamine hydrochloride

The hydroxylamine hydrochloride reagent is conventional reagent for determination carbonyl groups. The oxidized β-glucan reacts with this reagent to form the corresponding oxime. In addition, from data of carbazole assay it can be seen that 49-61% of the oxidation occured at C-6, producing aldehyde groups. This meant that 39-51% of the ketone groups were distributed between C-4 and/or C-2.

Earlier investigations of β-(1→4)-glucan (Bredereck K. 1967) indicated that, when cellulose derivatives were oxidized with this reagent, the favored attack was C-2, and that very little diketone is formed, but investigations with this reagent on native (1→3)-β-glucans had not previously been performed.

CONCLUSIONS

The results of the above paper show that β-glucan can be isolated from a *Saccharomyces cerevisiae* in a very pure form by the method used in this study. Thus, structural analysis gives reliable results. The structural characterization of the pure product was performed using the common analytical procedures: acid hydrolysis, methylation analysis combined with gas-liquid chromatography and gas-liquid chromatography-mass spectral analyses, enzymes hydrolyses and spectral anayses (FTIR, ^{1}H- and ^{13}C-NMR spectroscopy). On the basis of the obtained results, it was concluded that investigated glucan is sparsely branched, i.e. containes single β-D-glucopyranosyl units on every eight main chain unit which consists of (1→3)-linked β-D-glucopyranoses. No evidence was found for linkages of any other type.

In this work the conversion of some hydroxyl groups of purified β-glucan to aldehyde and ketone groups in reaction with methyl sulfoxide-acetic anhydride was investigated. Accompanyng the oxidation was the formation of (methylthio)methyl groups at a degree of substitution smaller than the degree of substitution for the carbonyl group. The total content of carbonyl group (aldehyde + ketone) is reflected by the increase in nitrogen content, by elemental analysis, after reaction of the respective samples of oxidized β-glucan with hydroxylamine reagent. The content of sulfur is also obtained from elemental analysis. The carbonyl groups which generated along the β-glucan chain were

confirmed by spectral data from the FT-IR spectra of oxidized β-glucan as well as by conversion into the polyoxime. The (methylthio)methyl ether groups can be removed by hydrolysis with weak acid. In this way a polysaccharide was obtained which retained glycosidic linkages. Some of the glucose units were converted into specified, keto- and/or aldehyde hexosyl residues which are the subject of further investigations.

Further work will concern the physiological effect of oxidized glucan in comparision to the native glucan. The structural requirements for example for an immunostimulation in humans or animals are still under discussion.

Because the source of this valuable polysaccharide is quite common and abundant, more attention should be paid to utilization of this microbial source in polysaccharide biotechnology.

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(1→3)-β-D-GLUKAN IZ *SACCHAROMYCES CEREVISIAE*: DOBIJANJE, KARAKTERIZACIJA I HEMIJSKA MODIFIKACIJA UVODJENJEM KARBONILNIH GRUPA U POLISAHARIDNI NIZ

Djordje ZEKOVIĆ[1], Milanka RADULOVIĆ, Aleksanda NASTASOVIĆ, Miroslav M. VRVIĆ[2,3] i Dragica JAKOVLJEVIĆ[2]

[1]"Alltech-Fermin", Senta
[2]Centar za hemiju, Institut za hemiju, tehnologiju i metalurgiju, Univerzitet u Beogradu, Beograd
[3]Hemijski fakultet, Univerzitet u Beogradu, Beograd, Srbija

Izvod

Brojni polisaharidi sa β-glikozidnom vezom rasprostranjeni su u različitim izvorima. Svi imaju opštu strukturu u kojoj je (1→3)-β-D-glukan suštinska gradivna jedinica. Oni privlače pažnju zato što su bioaktivni i imaju medicinske osobine, koje su bile opisane pre više od 50 godina.

Glukan je izolovan u čistom obliku i okarakterisan iz pekarskog kvasca *Saccharomyces cerevisiae* kao tehnološki važnog proizvoda. Podaci dobijeni pomoću opštih analitičkih metoda i potvrdjeni GLC-MS i NMR spektrima, ukazuju da izolovani glukan iz *Saccharomyces cerevisiae* ima kao osnovni gradivni niz (1→3)-β-D-povezane glukopiranoze sa pojedinačnim β-D-glukopiranoznim jedinicama u tačkama račvanja na svakih osam jedinica glavnog lanca.

Oksidativna transformacija β-D-glukana ispitivana je sa reagensom dimetil sulfoksid-anhidrid sirćetne kiseline, pri čemu su dobijeni keto-aldehido polimeri. Oksidaciju prati nastajanje (metiltio)metil grupa duž polisaharidnog niza, a stepen supstitucije je manji nego za karbonilnu grupu. Pošto su ove grupe acido-labilne, utvrdjeno je da mogu da se eliminišu kiselom hidrolizom, bez uticaja na sadržaj karbonila.

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