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OCCURRENCE OF (2-ACETAMIDO-2-DEOXYGLUCO)MANNANS IN THE CELLS OF CERTAIN YEASTS*

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ABSTRACT

D-Mannose-containing polysaccharides containing up to 6% of protein were obtained by extraction of several different yeasts with hot aqueous alkali followed by purification via their copper complexes. Their p.m.r. spectra show signals for *N*-acetyl groups arising from (2-acetamido-2-deoxy-D-gluco)-D-mannans, which are present in proportions up to 17%. The polysaccharides from *Pichia bovis* and *Saccharomyces phaseolosporus* contain 2-acetamido-2-deoxy-D-glucopyranosyl non-reducing end-units, as shown by methylation–fragmentation analyses. The residues in each polysaccharide appear to have the α-D-configuration.

INTRODUCTION

The cell walls of the majority of yeasts contain chemically bound, D-mannose-containing polysaccharides that can be liberated by the action of hot aqueous alkali. After purification of the products via the water-insoluble copper complexes formed with Fehling solution, their p.m.r. spectra show well-defined H-1 signals at τ 4.0–5.0 that can be utilized as a fingerprint[1] for identification and classification of yeasts (for a summary see ref. 2). The H-2–H-6 signals (τ 5.0–6.0) are less well-defined. Polysaccharides that contain 6-deoxyhexose residues, such as the L-rhamno-D-mannans from *Ceratocystis* spp[3], give sharp C–CH_3 doublets at τ 8.0–8.2.

A number of D-mannose-containing polysaccharides from yeasts give one or more signals at approximately τ 7.4. These signals, which occur in 18 of 410 different types examined in a survey[2], are indicative of the NAc groups of 2-acetamido-2-deoxyhexose residues. In the present study these residues are characterized as 2-acetamido-2-deoxyglucose units in each polymer, and as α-D-linked, non-reducing pyranosyl end-units in those from *Pichia bovis* and *Saccharomyces phaseolosporus*.

RESULTS AND DISCUSSION

The accurate chemical shifts of the *N*-acetyl signals for the 18 species of yeast that gave rise to p.m.r. signals at approximately τ 7.4 are listed in Table I, together

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TABLE I

THE PERCENTAGE CONTENTS OF PROTEIN, *N*-ACETYLATED RESIDUES, AND 2-AMINO-2-DEOXYHEXOSE IN (2-ACETAMIDO-2-DEOXY-GLUCO)MANNANS FROM YEASTS

	Recoverable protein in polysaccharide, %	*Chemical shift of* N-*acetyl signal(s)* (τ)	N-*acetylated units in polysaccharide* (%)[a]	*Approximate % of units containing 2-amino-2-deoxyglucose in polysaccharide*[b]
1 *Saccharomyces lactis* NRRL Y-1140	3.5	7.39	10	4
2 *Pichia bovis* UCD 60-20	4.6	7.34 (minor) 7.38	10	6
3 *Hansenula bimundalis* var. americana 506-67	3.8	7.34 7.38	5.9 4.0 } 10	4
4 *Pichia salictoria* UCD 61-344	3.5	7.39	6	5
5 *Pichia* sp. 3R-38-68[c]	4.7	7.34 7.38	4.9 3.8 } 9	6
6 *Saccharomyces dobzhanskii*	4.0	7.34	6	5
7 *Saccharomyces sociasi* CBS 4574	6.6	7.34	11	7
8 *Saccharomyces drosophila* PRL Y-116	3.5	7.34	13	4
9 *Hansenula fabianii* NRRL Y-1871	3.3	7.38	5	4
10 *Hansenula beijerinkii* YB4818 (or JPV 182 ?)	1.3	7.34	5	4
11 *Saccharomyces phaseolosporus* NRRL Y-1975	4.5	7.34	17	7
12 *Hansenula ciferii* NRRL Y-1031	1.5	7.33 7.38	1.5 1.3 } 3	2
13 *Saccharomyces wickerhamii* NCYC 546	3.1	7.33	5	3
14 *Hansenula saturnus* NRRL Y-1304	1.1	7.34 (trace) 7.39	3	2
15 *Candida freyschussii* CBS 2162	1.0	7.39	2	0.1
16 *Hansenula mrakii* NRRL Y-1364 and Y-6520	1.8	7.34 (trace) 7.39	3	3
17 *Pichia strassburgensis* 3R73-68	1.0	7.38	2	1
18 *Candida pelliculosa* NCYC 471	3.9	7.33	5	2
19 *Torulopsis stellata* NCYC 486	7.0	—	0	1

[a]As estimated by p.m.r. spectroscopy. [b]As estimated by the amino acid analyzer method, the values being corrected for a 55% sugar recovery. [c]Its polysaccharide has a p.m.r. spectrum identical with that of *P. bovis* in the H-1 region.

with the parent yeasts. The protein contents of the polysaccharides were low, ranging from 1.0 to 6.6%.

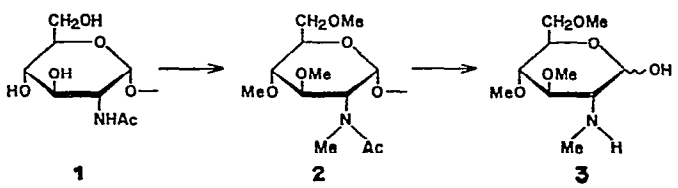
The polysaccharides from yeasts Nos. 1–13 (Table I) were hydrolyzed by acid, giving material identified paper-chromatographically as 2-amino-2-deoxyglucose by its R_F value and color reaction, and by degradation with ninhydrin to give an arabinose.

Yeasts Nos. 14–19 gave polysaccharides relatively low in amino sugars, and the 2-amino-2-deoxyhexoses in the hydrolyzates could not be detected on paper chromatograms because of streaking. However, when the hydrolyzates (and those from yeasts Nos. 1–13) were resolved by a Spinco Model 120C amino acid analyzer, a peak corresponding to 2-amino-2-deoxyglucose was obtained. This was distinguishable from 2-amino-2-deoxymannose and 2-amino-2-deoxygalactose.

The size of the peak obtained in the analysis was used to indicate the approximate percentage total of amino sugar residues present in the parent polysaccharide. The figure was based on a recovery of 55%, which is the amount of 2-amino-2-deoxy-D-glucose obtained on hydrolysis of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside hydrochloride under identical conditions. As shown by Foster *et al.*[4] methyl 2-amino-2-deoxy-α-D-glucopyranoside is more resistant to hydrolysis than its *N*-acetyl derivative. Under identical hydrolytic conditions the latter gave the free hexosamine in 35% yield.

The approximate figures for hexosamine release from the polysaccharides presented in Table I were compatible (in all but 2 cases–yeasts Nos. 15 and 19) with each amino sugar residue being *N*-acetylated. The percentage of *N*-acetylated residues in each polysaccharide was calculated accurately by comparison of one third of the area of the NAc signal with that of the H-1 signal.

The structures of D-mannose-containing polysaccharides from *P. bovis* and *S. phaseolosporus* were examined in more detail. Both polymers gave the D-enantiomorphs of mannose and 2-amino-2-deoxyglucose on hydrolysis and both were monodisperse in aqueous solution, giving single peaks on ultracentrifugation. Information on the structure of the 2-acetamido-2-deoxyglucose residues (**1**) was obtained by partial methylation by the Haworth procedure[5] followed by complete methylation by the Kuhn method[6]. The latter procedure causes *N*-methylation[7] in addition to *O*-methylation, and leads to structure **2**. Acid hydrolysis gave a mixture in which a 2-deoxy-2-methylamino-3,4,6-tri-*O*-methylglucose (**3**) was detected as a



1 **2** **3**

ninhydrin-positive spot on a paper chromatogram. Its R_F value corresponded to authentic material and was higher than those of mono-*O*- and di-*O*-methyl derivatives of 2-deoxy-2-(methylamino)glucose. Peaks were obtained using a Spinco amino acid

analyzer with retention times corresponding to the 3,4,6-tri-*O*-methyl- and a small proportion of the 3,6-di-*O*-methyl derivatives of 2-deoxy-2-methylaminoglucose[8]. The above methylation procedure was checked by using methyl 2-acetamido-2-deoxy-α-D-glucopyranoside[9] as starting material. The end product again corresponded to 3 on a paper chromatogram and gave a p.m.r. spectrum identical with that of an authentic specimen[8].

Since the above data obtained on the polysaccharides could be consistent with 2-acetamido-2-deoxy-D-glucofuranose end-units, the polysaccharides were treated with 0.1M hydrochloric acid at 100°, conditions that should remove these groups[10]. However, no monosaccharides were formed, confirming the original structural assignment.

The configurations of the 2-acetamido-2-deoxy-D-glucopyranose end-units should be predominantly α in polysaccharides of *P. bovis* and *S. phaseolosporus*, since the specific rotations of the polysaccharides are +103° and +107° respectively. These readings are higher than the rotations generally observed +80–90° with α-linked D-mannans[11,12].

The present data indicate the presence of (2-acetamido-2-deoxy-D-gluco)-D-mannans in the cell walls of a number of yeasts. In the polymers from *P. bovis* and *S. phaseolosporus* most of the 2-acetamido-2-deoxy-α-D-glucose residues are terminal and non-reducing. The locations of the amino sugar residues thus differ from that in the glycoprotein of baker's yeasts, in which the cell component contains a very minor 2-amino-2-deoxyglucose constituent, which serves as one of the bridges between the mannan and protein portions[13,14].

EXPERIMENTAL

Isolation and p.m.r. spectroscopy of polysaccharides. — The procedures for growth, isolation, and extraction of yeast cells are those described by Gorin and Spencer[1]. The extracted polysaccharides were purified via the insoluble copper complexes formed with Fehling solution. P.m.r. spectroscopy was carried out[1] with a Varian 100 MHz n.m.r. spectrometer by using 10% polysaccharide solutions in deuterium oxide at 70°. Tetramethylsilane in a coaxial capillary served as external standard ($\tau = 10.00$).

The percentages of *N*-acetyl groups in the polysaccharides were estimated by comparison of the area of the acetyl signal(s) at *ca.* τ 7.4 with that of the H-1 signals at τ 4.0–5.0.

Characterization of 2-acetamido-2-deoxyglucose residues in polysaccharides. — Polysaccharides were hydrolyzed with 3M hydrochloric acid for 3.5 h at 100° in evacuated, sealed tubes. Following evaporation, the hydrolyzates were examined on paper chromatograms, with 5:5:4 (v/v/v) isoamyl alcohol–pyridine–water as solvent[15] and *p*-anisidine hydrochloride as spray reagent. 2-Amino-2-deoxy-D-glucose was detected by a yellow spot differing in R_F value from 2-amino-2-deoxygalactose and 2-amino-2-deoxymannose. Some of the polysaccharides containing higher proportions

of amino sugar gave 2-amino-2-deoxyglucose in amounts sufficient for degradation with ninhydrin. The hydrolyzate (usually 50–60 mg) was dissolved in M sodium acetate–acetic acid buffer (pH 5.5, 25 ml) containing excess ninhydrin (200 mg) and hydrindantin (50 mg). After occasional stirring for 2 h at 70° the mixture was desalted on a Dowex-50 × 8 (H^+) column (2.0 × 20 cm). The product was examined on a paper chromatogram[16]; spray, *p*-anisidine hydrochloride; solvent, 40:11:19 (v/v/v) butyl alcohol–ethanol–water. Under these conditions 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose were degraded to an arabinose and a lyxose respectively*. Arabinose was obtained in each degradation, but was not formed from mannose. Mannose was detected[16] on paper chromatograms following hydrolysis in M sulfuric acid at 100°.

A more sensitive detection technique was used when only small proportions of amino sugar were present in the polysaccharides. Hydrolyzates were examined for amino sugar and amino acid content by using a Spinco Model 120C amino acid analyzer with the UR-30 type resin and a running time of 250 min (see Spinco Procedures Manual 120C-PM-1). Under these conditions 2-amino-2-deoxyglucose (retention time, r.t., 200 ±3 min) was eluted prior to 2-amino-2-deoxygalactose (r.t. 222 ±3 min)[18]. 2-Amino-2-deoxymannose had an intermediate r.t. of 210 ±3 min In each hydrolyzate a component having the r.t. of 2-amino-2-deoxyglucose was detected. Co-chromatography with known amounts of standard was carried out as a further check on the identity of the sugar, since the r.t.s. of the peaks were easily changed on slight variation of the operating procedures. The observed increase in peak height corresponded to the amounts of 2-amino-2-deoxyglucose added.

To find the approximate recovery of amino sugar from polysaccharide, methyl 2-acetamido-2-deoxy-α-D-glucopyranoside hydrochloride was hydrolyzed and estimated in a similar manner. A recovery of 55% of 2-amino-2-deoxyglucose was obtained, based on the colorimeter response of the Spinco analyzer. Methyl 2-amino-2-deoxy-α-D-glucopyranoside was similarly hydrolyzed, giving a 35% recovery of aminohexose.

Structural analysis of (2-acetamido-2-deoxy-D-gluco)-D-mannans from P bovis *and* P. phaseolosporus. — The mannose-containing polysaccharides were isolated from cells of *P. bovis* and *S. phaseolosporus* by the method of Gorin and Spencer[1]. They had $[\alpha]_D^{25}$ +103° and +107° (*c* 0.2, water), respectively. The polysaccharides were centrifuged at 59,000 r.p.m. as 0.15% and 0.45% solutions in 0.5% sodium chloride. Single peaks were obtained. D-Mannose was obtained crystalline from each polysaccharide following hydrolysis with M sulfuric acid for 18 h at 100°. 2-Amino-2-deoxy-D-glucose was obtained by using 3M hydrochloric acid for 3 h at 100° and characterized as its *N*-benzyloxycarbonyl[19] derivative.

The polysaccharides (1.0 g) were methylated by the Haworth procedure[5]; the reaction mixture was neutralized and dialyzed, and methylation was completed by the

*This modification of the method of Stoffyn and Jeanloz[17] was developed so that the pentoses produced by ninhydrin oxidation were not exposed to alkaline conditions that could lead to epimerization.

Kuhn method[6] until the product, in a chloroform solution, showed negative hydroxyl absorption at *ca.* 3600 cm^{-1} in the i.r. Samples of the methylated polysaccharides (50 mg) were refluxed in 3% methanolic hydrogen chloride (5 ml) for 18 h, the solutions were evaporated, and hydrolysis was completed with 3M hydrochloric acid (5 ml) for 3 h at 100°. Paper chromatography of the residue obtained on evaporation, with 40:11:2:19 (v/v/v/v) butyl alcohol–ethanol–pyridine–water as solvent and ninhydrin as spray reagent gave a blue spot corresponding to 2-deoxy-2-methylamino-tri-*O*-methylglucose, which had a mobility (R_{Rh}) compared with rhamnose of 1.3. This differed from the 3 mono-(R_{Rh} 0.6 and 0.7) and 3 dimethyl (R_{Rh} 1.0) ethers of 2-deoxy-2-methylamino-glucopyranose[8].

Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside was converted into 2-deoxy-2-methylamino-3,4,6-tri-*O*-methyl-D-glucose hydrochloride under identical methylation–fragmentation conditions as above, except that the product was extracted from the Haworth methylation mixture by chloroform. It was recognizable by its paper-chromatographic properties and its p.m.r. spectrum in D_2O (τ 4.4, doublet, *J* 3 2 Hz, H-1; 5.89, 5.95, 6.13, OMe; 6.76, NMe).

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