A Study of *Saccharomyces cerevisiae* Cell Wall Glucans

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ABSTRACT

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Much research has been carried out over the years examining cell wall glucans from *Saccharomyces cerevisiae* and this study further examines aspects of the binding of (1→4)-α-D-glucan in the yeast cell wall, using a number of isolation techniques as well as monoclonal antibodies able to recognize a mixed (1→4)-α-D-glucan/(1→6)-β-D-glucan. Extraction of purified glucan, from *S. cerevisiae* cell wall, with 0.1N HCl, at 80°C for 6 h, released into the solution (1→4)-α-D-glucan and (1→6)-β-D-glucan as the major polysaccharides, along with an insoluble pellet highly enriched in (1→3)-β-D-glucan. The released (1→4)-α-D-glucan was composed of a high molecular size >100 kDa fraction (7.2% w/w) and a medium 5–50 kDa polysaccharide (10.2% w/w), with the (1→4)-α-D-glucan covalently bound to the (1→6)-β-D-glucan. The average molar ratio of the α:β glucan was 47: 53 in this mixed polysaccharide. The structure of this polysaccharide was different from the structure of plant starch or animal glycogen as monoclonal antibodies specific to yeast (1→4)-α-D-glucan/(1→6)-β-D-glucan did not recognize the plant starch or animal glycogen standards.

Key words: (1→4)-α-D-glucan, 1→6)-β-D-glucan, glucan specific antibodies, NMR, *Saccharomyces cerevisiae*, yeast cell wall.

Abbreviations: BSA – Bovine Serum Albumin; D_2O – deuterium oxide (heavy water), d.w. – dry weight; DI – deionised water; GEM – Glucan Enriched Material, HRP – Horse Radish Peroxidase.

INTRODUCTION

The spent yeast from the brewing and distilling process has traditionally not been effectively utilized by the industry to produce high value products, despite the fact that this spent yeast is a reservoir of valuable compounds. The yeast cell wall in particular offers a plethora of possibilities. Much more research is still required regarding the purification and identification of the various yeast cell wall components, especially the yeast cell wall glucans. This paper focuses on just one area, the yeast cell wall glucans.

Glucans are prevalent among the *Saccharomyces cerevisiae* cell wall polysaccharides and the role of (1→3)-β-D-glucan in maintenance of yeast cell wall shape and rigidity[17,23] and the (1→6)-β-D-glucan as a polysaccharide that links together all of the cell wall polysaccharides is well documented[1,20] and reviewed[18]. The presence of soluble and insoluble glycogen-like (1→4)-α-D-glucan in the cells of *S. cerevisiae* grown aerobically, was frequently mentioned in the early yeast literature[12,14], and two forms of glycogen synthetase have been identified[13,28]. Glycogen is the energy reserve carbohydrate accumulated by *S. cerevisiae,* which can be mobilised during periods of yeast starvation. It is a polymer of α-D-glucose with a molecular weight of ~10^8 and a branched structure with 10–14 residues of α-D-glucose joined by 1→4 linkages[2,4].

The (1→4)-α-D-glucan content in the yeast cell wall can vary from as little as 1%[24] to as much as 29%[29] of the dry weight, depending upon the nutritional status of the cells, the method of isolation, the environmental conditions and the time the cells were harvested[24]. The industrially produced brewer's yeast cells described in a recent patent application[29] contained glucans at a 28.9% d.w. concentration, which included 12.4% d.w. of (1→4)-α-D-glucan. When these cells were treated with alkaline protease, the water insoluble cell wall contained 54.5% d.w. of glucans and more than half of the weight of (1→4)-α-D-glucan (29.2% d.w.). In 2002, Arvindekar and Patil[3] proposed an explanation for the presence of the insoluble fraction which has been defined by others as "difficult to wash away" yeast glycogen[11] within the yeast cell wall, based upon their finding that (1→4)-α-D-glucan is covalently bound to (1→6)-β-D-glucan. However, their discovery was in discord with the 1997 paper published by Kollar and co-workers[20] and with the recent paper published by Aimaniada and co-workers[1] on the role and the structure of the (1→6)-β-D-glucan within the yeast cell wall. These papers did not comment on the presence of (1→4)-α-D-glucan in the cell wall in a soluble or in a bound form[1,20]. If valid, the finding by Arvindekar and Patil[3] may add new clues to the elucidation of the bioactive components of the *S. cerevisiae* cell wall[33]. The bioactivity of (1→4)-α-D-glucans from microbial[10], fungal[15], plant[8,32] or animal[27] origin is well documented. Therefore, any additional research data that can confirm or deny the presence of (1→4)-α-D-glucan in a covalently bound form in *S. cerevisiae* cell wall might have, not only scientific, but also commercial value[29]. Arvindekar and Patil[3] worked with minute fractions from an enzymatic hydrolysis of the yeast cell wall and did not use NMR for the quantification or structural assignments for their separated products. Previous research in our laboratory has allowed for the same or very similar fractions, to be separated in

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large quantities from glucan enriched yeast cell wall preparations under weak acid hydrolysis conditions. To establish the ratios and carbohydrate composition of the separated polysaccharides, ^{1}H NMR has been found useful. The objective of this study was to examine the findings of Arvindekar and Patil[3] and to develop a practical, large scale separation method for this mixed (1→4)-α-D-glucan/(1→6)-β-D-glucan polysaccharide from *S. cerevisiae* cell wall.

METHODS AND MATERIALS

Yeast cell wall glucan preparation

An industrial grade (1→6)-β-D-glucan (ALL-BGY, Alltech Inc. Nicholasville KY) produced from *S. cerevisiae* by an enzymatic/alkaline/thermal treatment was washed 4× with DI water to remove any soluble residues. After freeze-drying, a 'glucan enriched material' (GEM) was obtained.

Monoclonal antibodies

Yeast (1→4)-α-D-glucan/(1→6)-β-D-glucan specific mouse monoclonal antibodies 513A431.1 (Alltech Inc., Nicholasville, KY, USA) and HRP labeled goat-anti-mouse antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) were used for ELISA testing. At the first stage of the monoclonal antibody preparation soluble *S. cerevisiae* yeast (1→4)-α-D-glucan/(1→6)-β-D-glucan/BSA conjugate was used for mouse immunization and for the selection of ascites and cell lines able to produce antibodies specific to the yeast cell wall (1→4)-α-D-glucan/(1→6)-β-D-glucan. Wheat starch, rice starch, potato starch, bovine glycogen, rat glycogen, oyster glycogen, and yeast β-glucan standard and all other reagents and materials were purchased from the Sigma Chemical Co. (St. Louis, MO, US).

Acidic digestion of yeast cell wall glucan

Figure 1, is a schematic that illustrates the preparation of the various fractions. The 'glucan enriched material' GEM (70 g) was subjected to hydrolysis with 700 mL of 100 mM HCl (pH 2.2) at 80°C for 6 h. After this time, the mixture was centrifuged at 13,500 × g/10°C/10 min and supernatant was collected. The pellet was extracted two times with 150 mL of DI water and freeze dried yielding 57.9 g of a white amorphous product (P1).

The two washes were combined with the original supernatant and neutralized to pH 7.0 with 2N NaOH. The precipitate that formed was collected (pellet P1/7) from the centrifugation (1.82 g after freeze-drying) and the supernatant was concentrated to a volume of 450 mL using a vacuum evaporator below a temperature of 37°C. This solution was further concentrated using 5 kDa Amicon® 15 ultra-filtration centrifuging devices (Millipore Corporation, Delaware USA).

The concentrate (~150 mL) was washed-centrifuged two times with two equal volumes of DI water, using the same ultra-filtering devices, to remove salt and the low molecular weight components resulting from the acid hydrolysis. The washed concentrate (S1) was freeze dried yielding 7.5 g of white amorphous product. A part of the pellet P1 was subjected to a second extraction using the

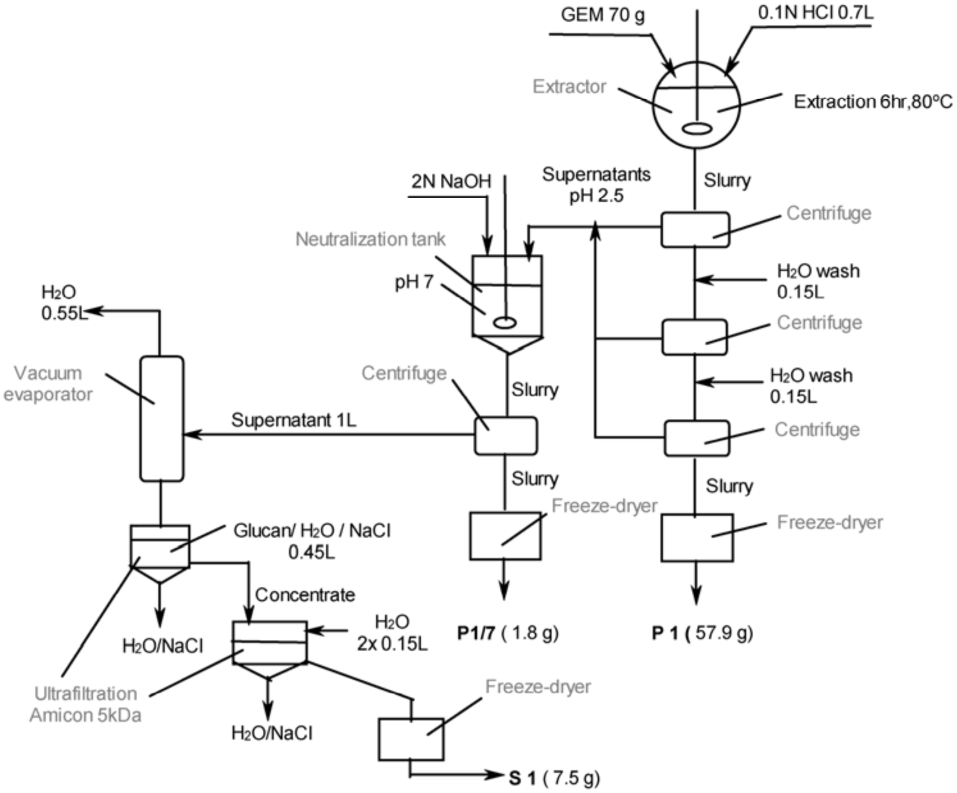

Fig. 1. A schematic of the production of glucan fractions P1,2,3 and S1,2,3.

same ratio of reagents and conditions as in the first 0.1N HCl extraction. These products were freeze-dried to yield P2 and S2.

The second extraction with 0.1N HCl did not produce a precipitate after the supernatant had been neutralized. A part of the pellet P2 was subjected to a third extraction using the same ratio of reagents and conditions as in the first 0.1N HCl extraction and the products were freeze-dried to yield P3 and S3.

The third extraction with 0.1N HCl did not produce a precipitate after the supernatant had been neutralized. The carbohydrate composition and the polysaccharide structure of the soluble and non-soluble fractions from the 0.1N HCl extractions were established by using ^1H NMR spectroscopy. The mannose and glucose content in the samples was analyzed using H_2SO_4-HPLC composition analysis[9]. The protein content was estimated using LECO combustion analysis and multiplying the nitrogen content by 6.25.

DEAE separation of S1 and S2 extracts

A DEAE cellulose-free base (Sigma E2145) was used for the chromatographic separations of the S1 and S2 extracts. This yielded better separation of sub-fractions than other cellulose based chromatography sorbents. A glass column (4.5 cm × 55 cm) packed with a DEAE slurry in 5 mM Na_2HPO_4 was employed. The 500 mg aliquot of S1 or S2 extract was dissolved in 5 mL of 5 mM Na_2HPO_4 and introduced to the top of the column and eluted with 5 mM Na_2HPO_4. Twelve 5 mL fractions were collected and filtered through 5 kDa Amicon® 15 ultra-filtration centrifuging devices to remove disodium hydrogen phosphate. The salt free samples were freeze-dried. The carbohydrate composition of the freeze-dried samples was estimated by comparing integrals for anomeric hydrogen in the ^1H NMR spectra (10 mg/mL of d^6-DMSO at 80°C).

Concanavalin A (Con A) chromatography

Fractions S1 and S2 were combined for this work to provide sufficient carbohydrate material for analysis after Con A separation since the fractions had an almost identical composition. A solution of 35 mg of the combined fractions S1 30–50 kDa and S2 30–50 kDa was prepared in 1 mL of Con A buffer (1N NaCl, 0.1M $CaCl_2$, 0.1M $MgCl_2$, 0.1M $MnCl_2$) and subjected to affinity chromatography on 15 mL of Concanavalin A coated Sepharose 4B placed in a glass column (1 cm × 15 cm) and eluted with Con A buffer.

Seven fractions of 5 mL were collected and the elution buffer was replaced by the wash buffer containing α-methylglucoside at a 0.5 M concentration. Seven more fractions of 5 mL were collected and concentrated using the 5 kDa Amicon® 15 ultra-centrifuging device. Each of the fractions was washed five times with 10–12 mL of DI water to remove all of the low molecular weight components. Intensive washing was necessary to remove the Mn^{2+} ions as these are paramagnetic and can broaden lines in the NMR spectra. All fractions were freeze-dried and the carbohydrate composition determined on the basis of ^1H NMR spectra integration.

NMR spectra

The NMR spectra were recorded using a Varian 400 MHz instrument. Samples (8–10 mg) were dissolved in 800 µL of D_2O or in d^6-DMSO /D_2O (10:1 w/w) mixture and recorded at 80°C, to stimulate efficient H/D exchange in the hydroxyl groups of the polysaccharide, and to shift the HOD signal outside the anomeric proton region. The chemical shifts of sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate, trimethylsilyl group signal was used as the 0 ppm standard. Interpretation of spectra was based on sets of chemical shifts for (1→4)-α-D-glucan and (1→6)-β-D-glucan standards from the literature[10,16,,20,26,28,31] NMR was used as fast and reliable diagnostic technique[5] to quantify the polysaccharides of interest, as it can be used in place of the less precise and laborious "wet" methods. It was not our intention to study a detailed structure of the researched polysaccharides with NMR at this time and therefore the set of chemical shifts presented in Table I from the literature were employed.

ELISA assay

The following materials (2 µg/mL) were tested using the ELISA protocol[7] for their ability to interact with monoclonal mouse antibody at a 1:100 dilution. The materials were: soluble glucan (>100 kDa), soluble glucan (10–100 kDa), S1 (>100 kDa), S2 (5–30 kDa), wheat starch, rice starch, soluble potato starch, bovine glycogen, rat glycogen, soluble oyster glycogen and a standard of (1→6)-β-D-glucan from *S. cerevisiae.* A goat-anti-mouse HRP at a 1:10,000 dilution in phosphate buffered saline was used as a secondary antibody detection method. Reactions were stopped after 6 min with 100 µL/well 1N HCl, and the plates were read at 450 nm using an automated microtitre plate reader (Labsystems, iEMS-MF, Finland).

Table I. Chemical shifts (ppm) of anomeric protons in (1→4)-α-D-glucan and (1→6)-β-D-glucan.

Glucan	H-1	H-6α	H-1 D_2O	H-6α D_2O	H-1 DMSO/D_2O	H-6α DMSO/D_2O	Literature reference
4-α-Glcp linear	5.38		5.36		5.08/5.14		10
t→4-α-Glcp terminal	5.38	3.80	5.36				10
4,6-α-Glcp branching	4.95		4.97		4.79		10
6-α-Glcp linear	5.05	3.85	5.05		4.97		10
3-β-Glcp linear	4.75	3.91	4.78		4.56	3.75	20, 31
3,6-β-Glcp branching	4.55		4.56		4.38		1, 10, 28
6-β-Glcp linear	4.52	4.20	4.52	4.21	4.30	4.03	1, 16, 20, 26, 31
t→3-β-Glcp terminal	4.63	3.91	4.64		4.42		16, 20, 26, 31
t→6-β-Glcp terminal	4.71	3.90	4.73		4.47		1, 16, 20, 26, 31
3-β-Glcp reducing end							
α	5.22	3.92	5.23		5.02		20, 31
β	4.66	3.89	4.68		4.51		20, 31

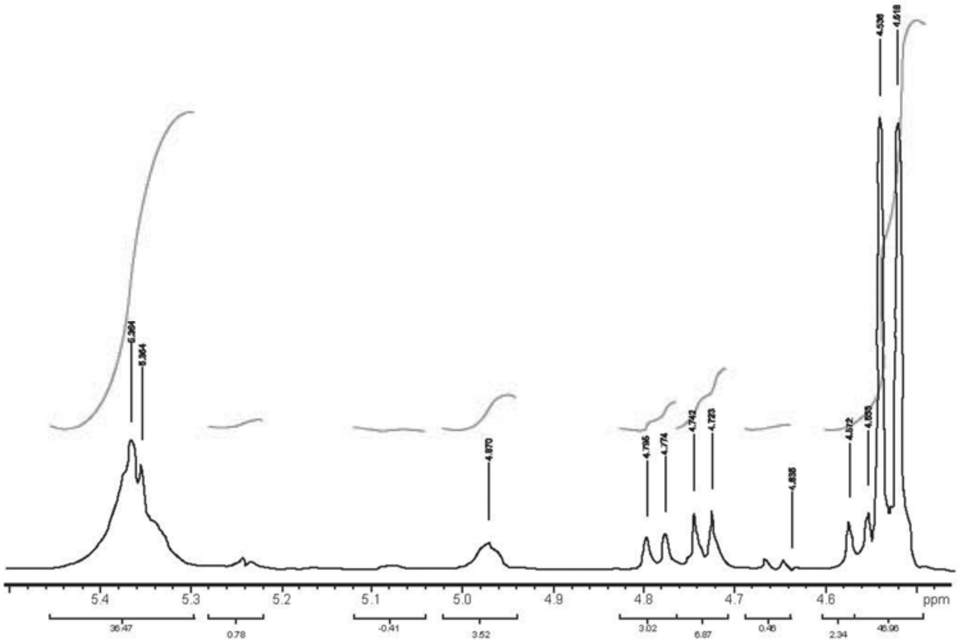

Fig. 2. The ^{1}H NMR spectrum (D_2O, 10 mg/mL, 80°C) of fraction S2 (50–100 kDa).

Table II. Composition of the freeze-dried products from 0.1N HCl extraction of 'glucan enriched material' (GEM).

Fraction	Weight[a]	Moisture %	Ash %	Protein %
GEM	100.00	0.24	4.7	5.95
P1	82.67	0.21	0.93	6.79
P2	82.68	1.28	0.33	7.19
P3	85.76	1.17	0.21	7.81
S1	10.71	0.21	3.95	2.02
S2	12.37	0.39	11.9	1.68
S3	8.26	0.10	28.42	3.48

[a] Calculated per 100 g of material taken for extraction

RESULTS

^{1}H NMR spectra

Sharp well defined non overlapping lines were found in the ^{1}H NMR spectra in D_2O of the water soluble extracts, allowing integration with little error. However, the spectra of the water insoluble fractions (pellets P1–P3), recorded in DMSO/D_2O, were more difficult to integrate because of partially overlapping signals. As an example of well resolved signals, the ^{1}H NMR spectrum in D_2O, of the anomeric protons of the S2 sub-fraction (50–100 kDa) is presented in Fig. 2.

Using NMR, the minute quantities of α-mannan that were present in the samples were not quantifiable and therefore α-mannan was not included in the polysaccharide balance. The relative content of chitin in the samples could be estimated by integration of the methyl signals from the CH_3CONH- groups of chitin N-acetyl-2-aminoglucopyranose rings, appearing between 2.05 and 2.2 ppm[32]. No chitin was detected in the samples that were examined in this study.

Composition of the fractions from the HCl extraction

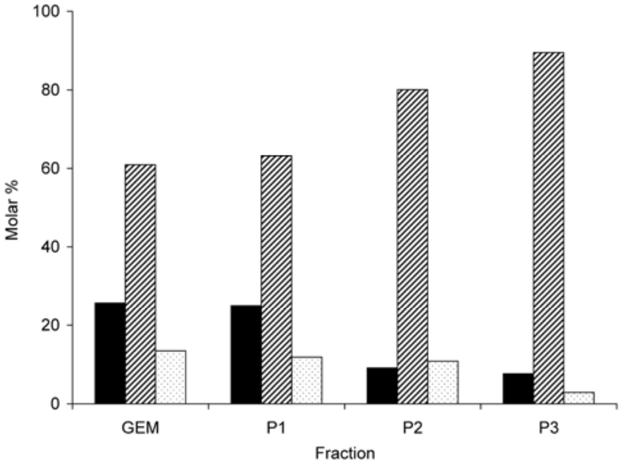

Fig. 3. Glucan composition of the 'glucan enriched material' (GEM) and of the pellets P1–P3 from the three subsequent 0.1N HCl extractions of this material: (1→4)-α-D-glucan (black bars), (1→3)-β-D-glucan (lined bars) and (1→6)-β-D-glucan (dotted bars).

The results of the three subsequent 0.1N HCl extractions of the glucan enriched material (GEM) from *S. cerevisiae* cell wall are given in Table II. The carbohydrate composition of the pellet changed with each of the extractions (Fig. 3) due to the soluble material that was removed. The relative content of (1→4)-α-D-glucan and (1→6)-β-D-glucan in the pellets decreased with each subsequent extraction concomitant with a relative increase in the insoluble (1→3)-β-D-glucan. The majority of the polysaccharides in the extracts survived 0.1N HCl treatment in the form of larger than 5 kDa molecules, and only a minor percentage on a weight basis, of the material subjected to hydrolysis (at 80°C for 6 h), was converted into the smaller oligosaccharides which permeated through the 5 kDa membrane and were discarded. It was speculated that at least part of the (1→4)-α-D-glucan that was solubilised during the HCl hydrolysis of the 'glucan enriched

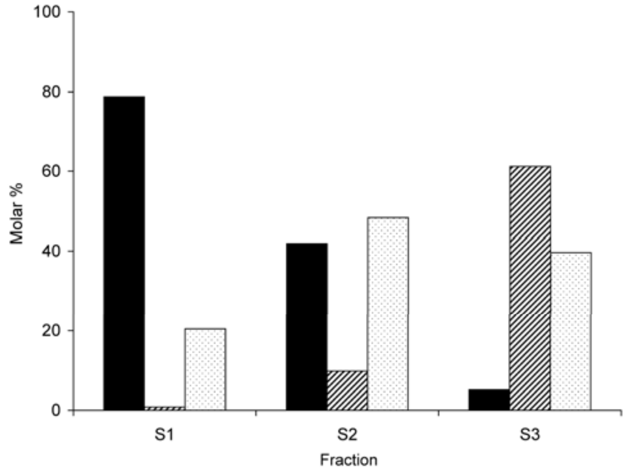

Fig. 4. Glucan composition of the supernatants S1–S3 from the three subsequent 0.1N HCl extractions: (1→4)-α-D-glucan (black bars), (1→3)-β-D-glucan (lined bars) and (1→6)-β-D-glucan (dotted bars).

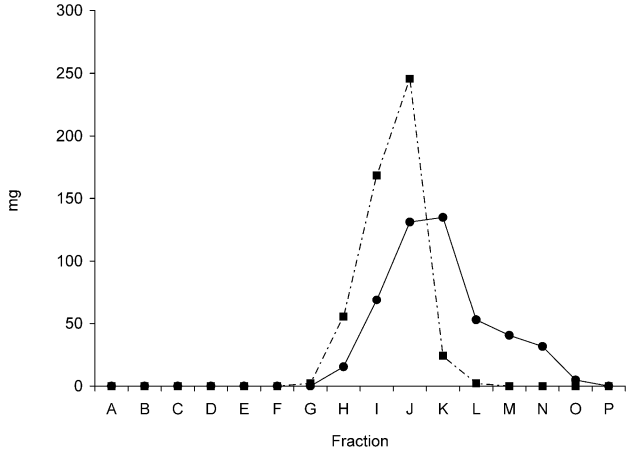

Fig. 5. DEAE ion exchange chromatography of S1 (●) and S2 (■) extracts from the 'glucan enriched material'.

material' would be still covalently bonded to the (1→6)-β-D-glucan. Previous experience in this laboratory using Amicon® 15 ultracentrifugation devices showed a good correlation between the size of the membrane pores and the molecular weight of the polysaccharides that permeated or were stopped during ultra-filtration[21]. These devices proved to be more practical for the removal of salts and low molecular weight organic molecules than the conventional dialysis membranes. Figure 4 illustrates the glucan composition of the supernatants from the extractions.

The (1→4)-α-D-glucan content in the first extract was significantly higher than the (1→6)-β-D-glucan, while in the second extract both polysaccharides were present in similar quantities. Such an observation might suggest that at least some of the (1→4)-α-D-glucan that was collected in S1 was not bound to (1→6)-β-D-glucan.

The third extraction released from pellet P2 an unexpectedly large quantity of (1→3)-β-D-glucan. This level constituted 61.2% of the carbohydrate present in supernatant S3.

DEAE chromatography of the S1 and S2 fractions

To ascertain if the mixed (1→4)-α-D-glucan/(1→6)-β-D-glucan was present in the yeast cell wall in a covalently bound form, DEAE ion exchange chromatography was conducted with the S1 and S2 fractions (Fig. 5). After purification and freeze drying the carbohydrate composition was estimated using ^{1}H NMR. Sixteen × 5 mL fractions (A-P) were collected. Figure 5 shows the elution patterns of both S1 and S2 fractions.

Fractions A-G contained no polysaccharides. Fraction H from the S1 extract contained predominantly (1→4)-α-D-glucan (90.9 mol%), while later fractions contained both (1→4)-α-D-glucan and (1→6)-β-D-glucan and a recovery of 96% was considered high. The S2 eluted as a narrow peak in only four of the 5 mL fractions and all four of those fractions contained both (1→4)-α-D-glucan and (1→6)-β-D-glucan (from 54.1% (1→4)-α-D-glucan in fraction H to 21.2% (1→4)-α-D-glucan in fraction K) with recovery close to 75%. An additional 16.7% of fraction S2 was collected as a (1→6)-β-D-glucan protein complex, when the column was rinsed with 320 mM Na_2HPO_4.

The elution patterns for both extracts were not identical. The 375 mg (75% w/w) of fraction S2 eluted as a narrow band in 20 mL of eluent, suggesting high homogeneity of the collected material. The 460 mg (96% w/w) of fraction S1 was collected in 45 mL of eluent, suggesting a much lower homogeneity of the S1 extract.

Molecular size distribution and glucan composition of the S1 and S2 fractions from the HCl extraction of the 'glucan enriched material'

A range of Amicon® 15 ultra-filtration centrifuging devices, with membranes of 100, 50, 30 and 5 kDa, were used to elucidate the molecular size distribution of 500 mg of fractions S1 and S2. The quantities and polysaccharide composition based upon ^{1}H NMR spectra integration of the fractions from ultra-filtration are displayed in Table III. The fraction S1>100 kDa contained predominantly (1→4)-α-D-glucan, while the fractions S1 30–50 kDa and S2 30–50 kDa contained almost equal quantities of (1→4)-α-D-glucan and (1→6)-β-D-glucan polysaccharides.

Con A chromatography of the combined fractions S1 30–50 kDa and S2 30–50 kDa

Fractions S1 and S2 (30–50 kDa) were combined for analytical purposes as they were very similar in regard to their carbohydrate composition and molecular size.

Unexpectedly, the first four void volume fractions contained 50.1% of the sample and were comprised of (1→4)-α-D-glucan/(1→6)-β-D-glucan (35/65) that did not adsorb to the Con A column. Three "empty" fractions followed the first four fractions. A change of the coating buffer to the wash buffer (containing a 0.5 M concentration of α-methylglucoside) released, in a single 5 mL aliquot, 42.9% of the (1→4)-α-D-glucan/(1→6)-β-D-glucan (69/31) that have been adsorbed on the Con A. Subsequent fractions that followed contained no detectable polysaccharides as determined by NMR. In total, the frac-

Table III. Size distribution and α/β-glucan composition of the S1 and S2 extracts based on ^{1}H NMR spectra integration.

Fraction	Weight (mg)	(1→4)-α-D-glucan (% w/w)	(1→6)-β-D-glucan (% w/w)	(1→3)-β-D-glucan (% w/w)
S1 5–30 kDa	54.0	58.5	36.8	4.7
S1 30–50 kDa	10.5	47.3	47.4	5.3
S1 50–100 kDa	23.0	56.2	40.9	3.0
S1 >100 kDa	340	83.5	15.3	1.2
S2 5–30 kDa	162	33.9	60.0	6.1
S2 30–50 kDa	45.0	46.4	50.4	3.2
S2 50–100 kDa	56.0	55.2	41.3	3.5
S2 >100 kDa	38.0	66.7	28.8	4.5

tions collected had a 93% recovery of the material that had been applied to the column.

From these results, it was hypothesized that the (1→4)-α-D-glucan needs to be present in large molar excess to secure interaction with the Con A lectin. Moreover, it is possible that there are different types of bonds between both of these polysaccharides and that some of these can inhibit complex formation between (1→4)-α-D-glucan and Con A. It was assumed that the fraction that has been adsorbed and washed from the Con A column with 0.5 M α-methylglucoside had the same properties as the analogous fraction collected by Arvindekar and Patil[3] and proven by them to be a mixed (1→4)-α-D-glucan/(1→6)-β-D-glucan yeast cell wall polysaccharide. The carbohydrate composition of this fraction, based upon its ^{1}H NMR spectrum, was 69% (1→4)-α-D-glucan: 31% (1→6)-β-D-glucan.

Interaction of yeast (1→4)-α-D-Glucan/(1→6)-β-D-Glucan specific monoclonal antibodies with samples from an acidic extraction of 'glucan enriched material' and plant starch and animal glycogen standards

Using the ELISA technique[7], mouse monoclonal antibodies specific to the yeast cell wall (1→4)-α-D-glucan/(1→6)-β-D-glucan were used to test for the presence of this glucan in the samples of soluble glucan >100 kDa, soluble glucan 10–100 kDa, S2 5–30 kDa and S1 >100 kDa. Controls included wheat, rice and potato starch, bovine, rat and oyster glycogen, as well as a sample of yeast β-glucan standard. A yeast glycogen control was not included as attempts to extract and purify the yeast glycogen to the level required for this test were not successful. Figure 6 shows interaction between the mouse monoclonal antibodies with these samples in the wells at a concentration of 2 μg/mL.

At the highest concentration of the antibody (1:100 dilution), none of the controls interacted with the yeast (1→4)-α-D-glucan/(1→6)-β-D-glucan specific antibody. The OD for the controls was < 0.04 for all tested, while for the samples containing the (1→4)-α-D-glucan/(1→6)-β-D-glucan, the OD values were all >0.86 suggesting an important structural difference between the yeast cell wall (1→4)-α-D-glucan/(1→6)-β-D-glucan and the (1→4)-α-D-glucan/(1→6)-β-D-glucan of the starch, glycogen and β-glucan used as controls.

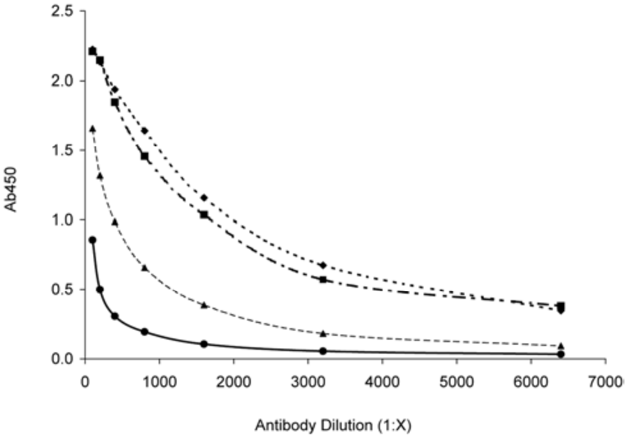

Fig. 6. Interaction of yeast (1→4)-α-D-glucan/(1→6)-β-D-glucan specific mouse monoclonal antibodies with samples from the acidic extraction of 'glucan enriched material'. Legend: (♦) soluble glucan 10–100 kDa; (■) soluble glucan >100 kDa; (▲) S1 >100 kDa; (●) S2 5–30 kDa.

DISCUSSION

These experiments have added several new clues to the evidence previously collected by Arvindekar and Patil[3] i.e., that *S. cerevisiae* cell wall contains a significant quantity of (1→4)-α-D-glucan connected through a covalent bond to (1→6)-β-D-glucan. The most important experiment in this respect was the ELISA testing of the sample of soluble glucan that was adsorbed on the Con A column and washed from it using 0.5 M α-methylglucoside solution. The (1→4)-α-D-glucan and the β-glucan controls used in the ELISA experiments were individual polysaccharide standards, which did not interact with (1→4)-α-D-glucan/(1→6)-β-D-glucan specific monoclonal antibodies, while the sample adsorbed on Con A and all of the samples of soluble glucan that were tested, showed a strong interaction with these antibodies suggesting that a chemical bond between (1→4)-α-D-glucan and (1→6)-β-D-glucan needs to be in place to cause this response in the ELISA tests.

Using an analogous sample, from a Con A separation, Arvidekar and Patil[3] suggested the existence of the chemical bond between these two polysaccharides. They used amyloglucosidase and lyticase to selectively hydrolyse these polysaccharides to examine their Con A sorption and to identify each of the components of this mixed (1→4)-α-D-glucan/(1→6)-β-D-glucan polysaccharide.

The large initial sample size in our work allowed the use of ^{1}H NMR to establish the carbohydrate composition of a variety of water soluble samples of yeast glucan and both of the polysaccharides of interest were always detected. However, there remains an important question to be addressed, why did Kollar and co-workers[20], who studied the role of (1→6)-β-D-glucan in yeast cell wall, not detect the presence of the (1→4)-α-D-glucan covalently bonded to the (1→6)-β-D-glucan? A possible answer is that they performed seven washings of the cell wall material and used 50 mM Tris-HCl and Tris buffer followed by incubation of the pellet with Zymolyase 100 T for 4 h at 30/37°C in 50 mM Tris-HCl buffer. They also did not mention affinity purification of the enzyme before use, to avoid α-glucanase activity, as was carefully done by Arvindekar and Patil[3].

In our opinion, the extensive washing and possibly, the use of a non selective Zymolyase, were the possible reasons for removal of the (1→4)-α-D-glucan/(1→6)-β-D-glucan that was present in the cell wall. What the biological reason is for the binding of (1→4)-α-D-glucan within the cell wall is still not clear, but one can assume that when it is bound it cannot be released outside of the cell wall and therefore it could be utilized as a source of energy under stress conditions.

The results of the 0.1N HCl hydrolysis of the 'glucan enriched material' demonstrated that the (1→4)-α-D-glucan/(1→6)-β-D-glucan was stable under the conditions of hydrolysis (i.e., 6 h at 80°C at pH 2.2), with only a minor portion of material being lost (less than 10%) in the form of molecules smaller than 5 kDa. Simplicity of the methods developed within this project make possible a large scale extraction of this (1→4)-α-D-glucan/(1→6)-β-D-glucan from 'glucan enriched material' produced from *S. cerevisiae* yeast cell walls.

In recent years, there has been increasing biotechnological and commercial interest in yeast cell wall components, including their use as biological response modifiers, anti-cancer agents, bioadsorbents, ingredients in food processing and cosmetic formulations, and as systems for immobilizing oral vaccines, antibodies and enzymes of industrial significance[6,25,33]. This increase in interest has occurred concomitantly with our better understanding of the chemical composition, genetic regulation and functional properties of cell wall components.

Recently, studies have demonstrated that these non-digestible carbohydrates play an important role in animal production[19,30] and human health[22], through their involvement in host cellular metabolism, protein structure and function, cell-to-cell communication and host immunity. The data generated in the multitude of animal health and production studies indicate that there is merit in establishing clinical studies to investigate these functional properties. A greater understanding of cell wall composition and component functionality may lead to the development of new food, feed and pharmaceutical applications. The brewing industry has the ability to supply the industry with raw material, spent yeast, or finished products with substantial financial return.

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