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Isolation and Composition of an Alkali-soluble Glucan from the Cell Walls of *Saccharomyces cerevisiae*

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SUMMARY

An alkali-soluble glucan was obtained from the cell walls of *Saccharomyces cerevisiae* NCYC1109 and baker's yeast by extraction with cold, dilute sodium hydroxide under nitrogen. The glucan, which represented approximately 20 % of the cell wall, precipitated as a gel when the alkaline extract was neutralized. The purified glucan was homogeneous and was shown to be free from contamination by other cell-wall polysaccharides by ultracentrifuging, gel filtration and electrophoresis. In addition to glucose, the glucan contained traces of mannose and nitrogen, but no hexosamine. Structural analyses revealed the presence of 80–85 % (1→3)-β-D linkages, 8–12 % (1→6)-β-D linkages and 3–4 % branched residues linked through C-1, C-3 and C-6. The molecular weight of the glucan was estimated to be about 250000. Electron-microscopic examination of the cell walls after alkali extraction showed that an amorphous surface layer had been removed revealing numerous bud scar structures.

INTRODUCTION

The biochemistry of the yeast cell wall has been comprehensively reviewed by Phaff (1963, 1971). The main cell-wall components of baker's yeast (*Saccharomyces cerevisiae*) are glucan and mannan type polysaccharides. Yeast mannan structure has been reviewed by Ballou (1974). Our previous studies have shown the presence of at least two types of glucan in baker's yeast cell walls: an alkali-insoluble, predominantly (1→3)-β-D-linked glucan (Manners, Masson & Patterson, 1973*a*); and an alkali-insoluble acetic acid-soluble, highly branched (1→6)-β-D-linked glucan (Manners *et al.*, 1973*b*; Manners, Masson & Patterson, 1974). However, reference has been made to the occurrence of a third glucan component in yeast cell walls, which, unlike the other two components, is extracted from the walls with alkali.

Roelofsen (1953) reported that approximately 25 % of the total cell-wall glucan of baker's yeast was extracted with 2 % sodium hydroxide at 100 °C. Kessler & Nickerson (1959) obtained an alkali-soluble glucan from the cell walls of *S. cerevisiae* using the milder extraction conditions of 1 M-potassium hydroxide at 30 °C under nitrogen. This glucan was complexed with protein and mannan. However, extraction of cell walls from the related yeast *S. carlsbergensis* with cold, 3 % sodium hydroxide under nitrogen yielded separate components of mannan and alkali-soluble glucan (Eddy & Woodhead, 1968). In this case, the glucan represented approximately 20 % of the initial cell-wall material.

These demonstrations that *S. cerevisiae* cell walls had an alkali-soluble glucan

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component were not accompanied by any structural analysis. Since this glucan may well be a major cell-wall component, we initiated a study to confirm its presence and determine its chemical nature.

METHODS

Organism. Saccharomyces cerevisiae NCYC1109 was maintained on 0·5 % yeast extract + 5 % glucose agar slants. Liquid cultures of the yeast were grown in 1 l portions of the same medium (without the agar) in 2 l conical flasks. Cultures were grown at 25 °C with shaking and cells in the late-exponential phase of growth were harvested by centrifuging. The cells were washed three times with 0·2 M-sodium phosphate buffer, pH 8·5, and frozen until required for wall preparation. Commercial baker's yeast was obtained from the Distillers Co. Ltd, Edinburgh.

Preparation and extraction of cell walls. Cell walls of *S. cerevisiae* NCYC1109 and of baker's yeast were prepared by mechanical disruption in a Braun homogenizer as described previously (Fleet & Phaff, 1973) except that sodium phosphate buffer, pH 8·5, was used in place of Tris buffer. After washing approximately 20 times with buffer, and three times with distilled water, the cell walls were freeze-dried and stored in a desiccator. The walls were then extracted according to the protocol in Fig. 1.

Analytical procedures. Total acid hydrolysis of glucan samples was done at 100 °C in 90 % (v/v) formic acid for 2 h, followed by M-H_2SO_4 for 3 h as described by Peat *et al.* (1958). Partial acid hydrolysis was carried out at 100 °C with 90 % formic acid for 1 h, followed by 0·16 M-H_2SO_4 for either 0·5 h or 1 h. Formic acid was effectively removed by adding water and evaporating the solution to dryness several times, before treatment with the H_2SO_4. Hydrolysates were neutralized with $BaCO_3$ and, where necessary, deionized with mixed Amberlite IR–120 and Dowex 1–X8 resins.

Reducing sugars were determined with an alkaline copper reagent (Spiro, 1966) and glucose was measured specifically with the glucose oxidase assay (Huggett & Nixon, 1957). Total hexose was determined by the phenol–sulphuric method of Dubois *et al.* (1956). Nitrogen was determined by the micro-Kjeldahl method and hexosamine was estimated by the procedure of Elson & Morgan (1933). For hexosamine analysis, the polysaccharides were hydrolysed with 4 M-HCl for 4 h under nitrogen at 110 °C. Neutral sugars were removed from the hydrolysate using Amberlite IR-120 resin, as described by Spiro (1966).

Descending paper chromatography was done with the following solvent systems: A, ethyl acetate–pyridine–water (10:4:3, by vol.); B, n-propanol–ethyl acetate–water (14:2:7); C, ethyl acetate–acetic acid–90 % formic acid–water (18:3:1:4). Sugar spots were detected using an alkaline $AgNO_3$ reagent (Trevelyan, Procter & Harrison, 1950). Column chromatography was carried out with either Sepharose 6B or Bio-Gel P-2 (200–400 mesh), as recommended by Pharmacia and Bio-Rad respectively.

The degree of polymerization (DP) of glucans was determined from the sorbitol content of an acid hydrolysate of the borohydride-reduced glucan by using sorbitol dehydrogenase (Manners, Masson & Sturgeon, 1971).

For ultracentrifuging (Beckman, Model E), the glucans were dissolved in either 1·5 % NaOH or 100 % dimethyl sulphoxide (DMSO) and subjected to sedimentation velocity analysis using Schlieren optics.

Polyacrylamide gel electrophoresis of polysaccharides was done using 4·6 % gels and 0·02 M-sodium tetraborate buffer, pH 9·5. Electrophoresis was conducted in the cold (4 °C) and at 300 V for 2 h. Gels were sliced into 2 mm sections, incubated overnight in 1·0 ml water and then analysed for total carbohydrate.

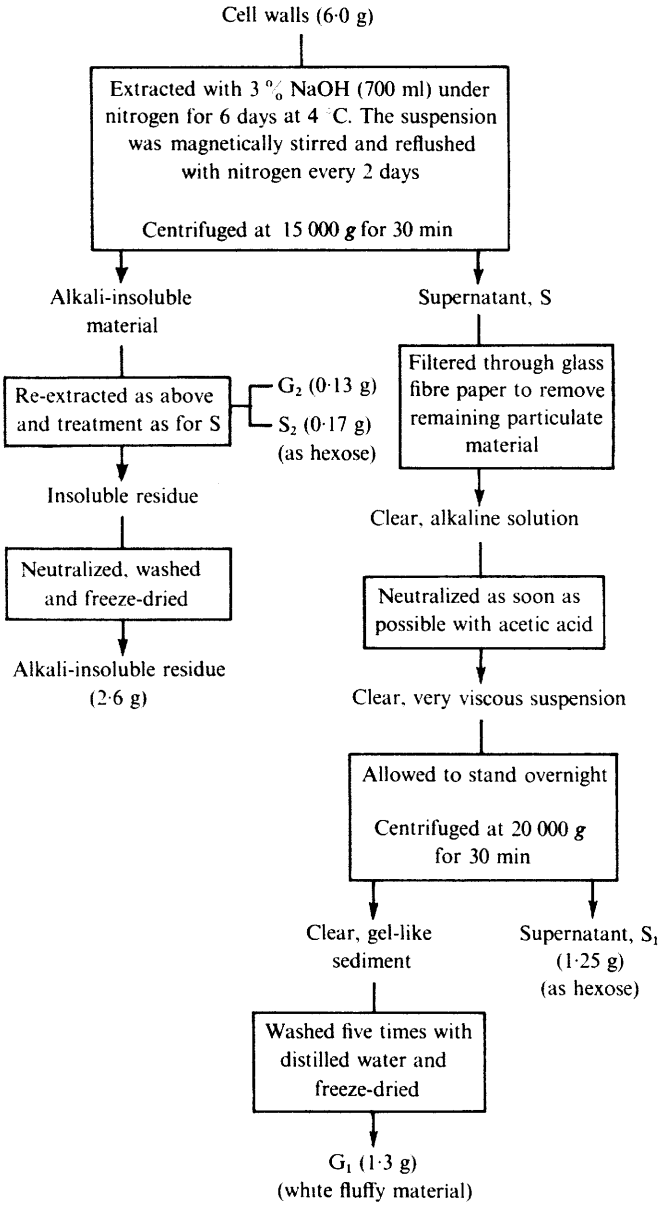


Fig. 1. Procedure for extracting *S. cerevisiae* cell walls. Baker's yeast cell walls (9·0 g) were similarly extracted (once only) giving 1·2 g of G_1 and 2·4 g of alkali insoluble residue. With this yeast, both G_1 and the insoluble residue failed to sediment firmly on centrifuging, resulting in lower yields.

Periodate oxidation analysis. The glucans were uniformly suspended in distilled water using a Potter homogenizer and concentrations were adjusted to 0·03 M-sodium meta-periodate and 2 mg polysaccharide/ml. Oxidations were conducted in the dark at 4 °C. Periodate reduction with time was monitored spectrophotometrically (Aspinall & Ferrier, 1957). For Smith degradation (Goldstein *et al.*, 1965), oxidation of the glucans (50 mg) was stopped after 96 h by adding ethylene glycol (1·0 ml) and the polysaccharide was

recovered by centrifuging. After washing, the material was resuspended in water (25 ml) and reduced with KBH_4 (0·15 g). Reduction was carried out for 24 h, and then the suspension was neutralized and made up to 0·1 M-H_2SO_4. Hydrolysis was carried out at 20 °C for 24 h with shaking. Residual insoluble glucan was then isolated by centrifuging, washed and freeze-dried. The supernatant solution was neutralized, concentrated by rotary evaporation, and used for either column or paper chromatography. The latter was preceded by deionization as above.

Methylation analysis. Polysaccharides were methylated by the Hakomori method following the recommendations of Lindberg (1972). The methylsulphinyl sodium reagent was prepared according to the procedure of Sandford & Conrad (1966), although clear, green solutions of the anion took about 2 h to form. Anion reagent (5 ml) was added to a solution of the glucan (20 mg) in dimethyl sulphoxide (5 ml). After shaking overnight at 20 °C under nitrogen, an excess of methyl iodide (5 ml) was added. The solution was allowed to stand for 5 to 6 h, and then the methylated glucans were poured into water (100 ml), extracted with chloroform (3 × 100 ml), washed with water, and evaporated to dryness. At this stage, the polysaccharide was washed with ethanol, to avoid interference in subsequent gas–liquid chromatographic analysis. The procedure removed contaminating by-products of the methylation process leaving a clear, alcohol-insoluble polysaccharide. The methylated glucan was dried for subsequent hydrolysis or remethylation as required. Total acid hydrolysis was carried out as described previously. The methylated monosaccharides were converted into their alditol acetates (Lindberg, 1972) for analysis by g.l.c using a Pye series 104 gas chromatograph fitted with a glass column (150 × 0·4 cm) packed with 3 % OV 225 on Gas Chrom Q (100–200 mesh). The methylated sugars were separated at 200 °C with nitrogen carrier gas at 20 ml min^{-1}. The identity of the methylated derivatives was confirmed by mass spectrometry (Bjorndal *et al.*, 1970) using an AEI mass spectrometer coupled to a gas chromatograph.

Electron microscopy. Cell-wall preparations were allowed to dry on to carbon-coated formvar grids which were then shadowed at an angle of 12° with platinum and examined in an AEI EM802 electron microscope.

Materials. Laminarin (Fleming, Hirst & Manners, 1966), luteose (Nakamura & Tanake, 1963), baker's yeast (1→6)-β-D-glucan (Manners *et al.*, 1973*b*), and pachyman (Hoffmann, Simson & Timell, 1971) were obtained from the departmental collection. Sclerotan was a gift from Dr J. S. D. Bacon (Jones, Gordon & Bacon, 1974). Pustulan was purchased from Calbiochem, and dextrans T-110, T-250, and T-500 were obtained from Pharmacia.

RESULTS

Wall extraction

The alkali treatment dissolved 57 % of the cell-wall material from *S. cerevisiae* 1109 (see Fig. 1). The yields obtained for the various fractions accounted for over 90 % of the initial wall material. The remaining 10 % was accounted for by losses in washing and by substantial amounts of protein present in the S_1 and S_2 fractions. After heating (100 °C for 5 min), fractions S_1 and S_2 reacted with Fehling's solution to produce dense flocculent precipitates, suggesting they had a high mannan content (Phaff, 1963). Precipitation with this reagent did not occur unless the fractions had been preheated. Fractions S_1 and S_2 were not studied any further. Fraction G_1 accounted for 22 % of the cell wall and is the subject of this investigation. Additional alkali treatment gave only minor amounts of G_2 which was not further examined. Baker's yeast G_1 was also prepared according to Fig. 1.

Table 1. *Chemical composition of cell-wall G_1 preparations*

Component	Composition (%) *S. cerevisiae* 1109	Composition (%) Baker's yeast
Carbohydrate	99·0	98·0
Nitrogen	0·13	0·26
Hexosamine	0·0	0·0
Glucose	98·5	97·0
Mannose	1·5	3·0

Solubility of G_1

The G_1 fractions from both yeasts were insoluble in water but readily soluble in M-sodium hydroxide and 100 % dimethyl sulphoxide (DMSO). Baker's yeast G_1 took longer to dissolve than the *S. cerevisiae* 1109 preparation, requiring 1 to 2 days at 37 °C in DMSO for complete dissolution. Neutralization of sodium hydroxide solutions of G_1 fractions with either acetic acid, glycine or hydrochloric acid produced gels at pH values below 10·0. However, if boric acid was used for neutralization, gelation did not occur until below pH 9·0, provided that the concentration of G_1 was not greater than 5 mg ml^{-1}.

Chemical analyses

Paper chromatographic analyses (solvent A) of total acid hydrolysates of G_1 from both yeasts revealed that they contained glucose plus minor amounts of mannose. Quantitative comparisons of specific glucose content and reducing-sugar equivalents of the hydrolysates showed 98·5 % glucose and 1·5 % mannose for the *S. cerevisiae* 1109 preparation, and 88·4 % glucose and 11·6 % mannose for baker's yeast G_1. Since the mannose in the latter hydrolysate could have been due to contamination from mannan present in the S_1 fraction, the G_1 preparations were washed with distilled water a further five times. The preparations were homogenized at each wash to ensure uniform suspension. The glucose and mannose contents of the washed fractions were then redetermined. There was no reduction in the mannose content of G_1 from *S. cerevisiae* 1109, but the mannose content of the baker's yeast preparation dropped to 3 %, suggesting there had been some initial contamination with mannan. This was consistent with the difficulties encountered in isolating and washing this fraction (Fig. 1). The final low mannose content of the washed G_1 fractions was not reduced by further washing and so was not considered to arise from S_1 contamination. The further washed G_1 fractions were used in all subsequent studies.

Table 1 shows the results of the chemical analysis of the G_1 fractions. Both preparations contained a small amount of nitrogen, but no hexosamine.

Partial acid hydrolysis

Paper chromatographic analyses (solvents A and B) of partial acid hydrolysates from G_1 fractions (20 mg) of either yeast showed the following components: glucose, laminaribiose, laminaritriose, gentiobiose, laminaritetrose, laminaripentose, gentiotriose, laminarihexose, gentiotetrose, plus higher unidentified oligosaccharides. The laminarisaccharides showed much higher spot intensities than the gentiosaccharides. Partial acid hydrolysates of laminarin and luteose were run simultaneously, as controls, together with the appropriate standards. The results suggested that fraction G_1 was predominantly a (1→3)-β-D-linked glucan containing a minor proportion of (1→6)-β-D linkages.

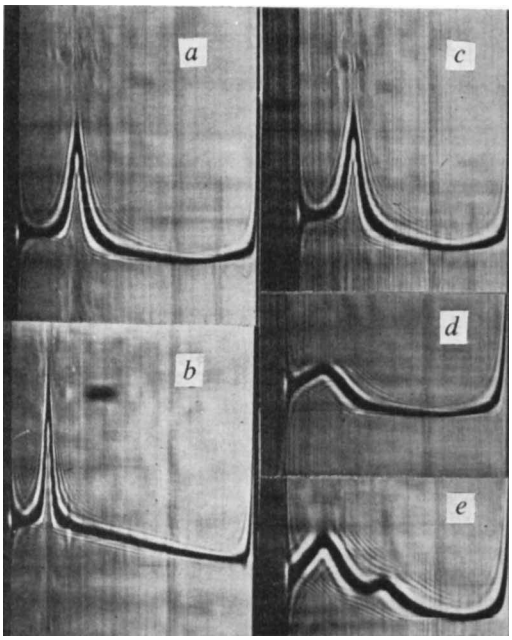


Fig. 2. Sedimentation patterns from ultracentrifuging of glucan samples. (*a*) *S. cerevisiae* 1109 G_1 in 1·5 % sodium hydroxide, 5 mg ml^{-1}, 53 min; (*b*) *S. cerevisiae* 1109 G_1 in 100 % DMSO, 5 mg ml^{-1}, 169 min; (*c*) Baker's yeast G_1 in 1·5 % sodium hydroxide, 5 mg ml^{-1}, 49 min; (*d*) Yeast (1→6)-β-D-glucan in 1·5 % sodium hydroxide, 3 mg ml^{-1}, 76 min; (*e*) Baker's yeast G_1, 3 mg ml^{-1} and yeast (1→6)-β-D-glucan, 3 mg ml^{-1}, in 1·5 % sodium hydroxide, 72 min.

Homogeneity studies

The small proportion of (1→6)-β-D linkages present in fraction G_1 could arise from contamination with the (1→6)-β-D-glucan described by Manners *et al.* (1973*b*), so the following experiments were conducted to investigate the possibility.

Ultracentrifuging of sodium hydroxide or dimethyl sulphoxide solutions of G_1 showed only one symmetrical boundary (Fig. 2). Figure 2 also shows the sedimentation patterns of baker's yeast (1→6)-β-D-glucan and a mixture of this glucan and G_1. At concentrations of 3 mg ml^{-1} in 1·5 % sodium hydroxide, *S. cerevisiae* 1109 G_1, baker's yeast G_1, and yeast (1→6)-β-D-glucan exhibited sedimentation coefficient values (*s*) of 9·3, 13·5 and 3·9 respectively. At 5 mg ml^{-1} (in 1·5 % sodium hydroxide) the *S. cerevisiae* 1109 preparation gave an *s* value of 8·35 and baker's yeast G_1 gave a value of 8·45, indicating the effect of solute concentration on sedimentation behaviour. A 5 mg ml^{-1} solution of *S. cerevisiae* 1109 G_1 in 100 % DMSO showed a much lower *s* value of 1·7.

As mentioned previously, G_1 preparations were maintained in solution at pH 9·5 if alkaline solutions of the polysaccharide were neutralized with boric acid. Such solutions were chromatographed on Sepharose 6B. Although preparations of G_1 were excluded from this column, no polysaccharide was detected in those fractions where the yeast (1→6)-β-D-glucan eluted (Fig. 3). However, on the basis of molecular size (see later), preparations of G_1 should have been included in this column, not excluded. Solutions of pachyman, sclerotan and barley β-glucan were similarly excluded. The reason for this unusual behaviour is not understood, but it is possibly associated with the conformational state of the molecule. A more

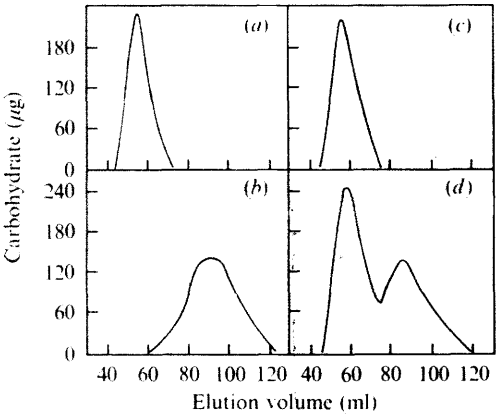


Fig. 3

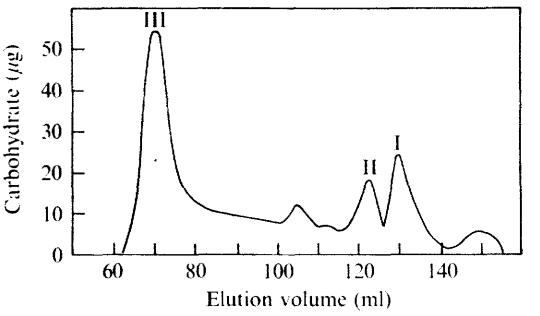


Fig. 4

Fig. 3. Sepharose 6B chromatography of the cell-wall glucan fractions: (*a*) Baker's yeast G_1; (*b*) Baker's yeast (1→6)-β-D-glucan; (*c*) *S. cerevisiae* 1109 G_1; (*d*) *S. cerevisiae* 1109 G_1 and Baker's yeast (1→6)-β-D-glucan. Approximately 5 mg of polysaccharide in 1·0 ml solvent was loaded on to the column (85 × 1·5 cm; V_t 150 ml; V_0 56 ml) which was equilibrated and eluted with 0·05 M-sodium tetraborate buffer, pH 9·5. Flow rate was 5·0 ml h^{-1} and fractions of 3·0 ml were collected.

Fig. 4. Bio-Gel P-2 chromatography of the soluble products from Smith degradation of the cell-wall G_1 preparation from *S. cerevisiae* 1109. The column (112 × 1·5 cm; V_t 200 ml; V_0 69·0 ml) was equilibrated and eluted with 0·05 M-NaCl. Flow rate was 6·0 ml h^{-1} and fractions of 3·0 ml were collected.

detailed study of the behaviour of these polysaccharides on gel chromatography has been published (Fleet & Manners, 1975).

Preparations of G_1 (boric acid-neutralized solutions) did not penetrate the polyacrylamide gels on electrophoresis. However, under the same conditions, the yeast (1→6)-β-D-glucan exhibited substantial movement (2·7 cm after 1 h) towards the anode. No polysaccharide was found in this area of the gel on electrophoresis of G_1 preparations. The smaller (1→3)-β-D-glucan, laminarin, migrated 5·4 cm after 1 h and the dextrans T-110 and T-500 migrated 0·9 cm and 0·3 cm respectively after 2 h. The (1→6)-β-D-linked glucans, pustulan and luteose (small molecular-weight molecules) did not enter the gels under the conditions used. In addition to molecular size, other factors influence the electrophoretic mobility of polysaccharides in polyacrylamide gels.

The above experiments show that the (1→6)-β-D linkages present in the G_1 preparations are not due to contamination by the other cell wall (1→6)-β-D-glucan, and that fraction G_1 is probably a homogeneous polysaccharide.

Molecular size

Using the sorbitol dehydrogenase method, the G_1 preparation from baker's yeast exhibited a DP value of 1810 and the *S. cerevisiae* 1109 preparation gave a value of 1330. Both values are considered to be accurate to within ± 10 %. Under the same conditions, the standard dextrans, dextran T-110, T-250 and T-500 gave DP values of 407, 877 and 1403 respectively, which correspond well with the M_n values of 62000, 141000 and 185000 published by the manufacturers.

Methylation analysis

Samples of fraction G_1 were fully methylated after three treatments using the Hakomori procedure. After only one methylation and subsequent hydrolysis and g.l.c. analysis, there

Table 2. *G.l.c.–methylation analysis of cell-wall G_1 preparations before and after Smith degradation*

O-Methyl-D-glucitol acetate derivative	Retention time	Type of linkage	Composition (mol %) *S. cerevisiae* 1109 *a*	*S. cerevisiae* 1109 *b*	Baker's yeast *a*	Baker's yeast *b*
2,3,4,6-Tetra-	1·00	End-groups	3·7	2·2	4·7	2·5
2,4,6-Tri-	1·67	(1→3)-	84·7	94·4	79·3	95·0
2,3,4-Tri-	2·00	(1→6)-	8·3	0·0	12·0	0·0
2,4-Di-	3·44	Branch point at C-1, C-3 and C-6	3·3	3·4	4·1	2·5

a, Undegraded preparations; *b*, Smith-degraded preparations.

was no evidence of any unmethylated or monomethylated glucitol derivatives, which are indicators of incomplete methylation. However, three methylations were required to eliminate a minor peak of 2,6-di-*O*-methyl-D-glucose (considered as an artefact of under-methylation) and to obtain ratios of tetra-*O*-methyl and di-*O*-methyl derivatives that were consistent with a fully methylated polysaccharide. The data in Table 2 show that fraction G_1 contains mainly (1→3)-linked residues, with occasional branching at C-6, and a small but significant percentage of (1→6)-linked residues. The baker's yeast preparation showed a slightly higher incidence of the latter type of linkage. The absence of derivatives of 2,3,6-tri-*O*-methyl-D-glucose from the hydrolysis products of the methylated glucans is evidence that the glucans are not contaminated with alkali-soluble glycogen (cf. Gunja-Smith & Smith, 1974). The methylated derivatives were identified by comparison with reference substances obtained from methylated polysaccharides of known structure (laminarin, Fleming *et al.*, 1966; pustulan, Hellerqvist, Lindberg & Samuelsson, 1968; sclerotan, Jones *et al.*, 1974) and confirmation by mass spectrometry.

Periodate oxidation analysis

Both G_1 preparations reduced only small amounts of periodate, which is consistent with a predominantly (1→3)-linked glucan containing a small percentage of (1→6)-linked residues. Periodate reduction (which was constant after 20 h) was 0·23 molecular proportions for the *S. cerevisiae* 1109 preparation, and slightly higher at 0·32 molecular proportions, as expected from the methylation studies, for the baker's yeast preparation. The contributions of the (1→6)-linked residues to the periodate reduction results (cf. Table 2) are calculated to be 0·16 and 0·24 molecular proportions respectively.

On Smith degradation, G_1 (50 mg) from *S. cerevisiae* 1109 yielded 27·4 mg of an insoluble, resistant fraction, suggesting the presence of long sequences of (1→3)-linked residues in the parent molecule. Since approximately 45 % of the original molecule dissolved, some of the periodate-sensitive (1→6) linkages must have been interspersed among sequences of (1→3) linkages. On concentration, the dissolved material gave rise to additional insoluble polysaccharide (7 mg). Paper chromatographic analysis (solvent C) of the soluble products showed mostly glycerol (R_{glc} 3·5) and glucosyl-glycerol (R_{glc} 1·0), with traces of glycollic aldehyde (R_{glc} 2·43) and slower-moving unresolved material. Column chromatography on Bio-Gel P-2 (Fig. 4) gave an excluded peak of carbohydrate (III) and several minor included peaks. Peak I was identified by paper chromatography as a mixture of glucosyl-glycerol and glycollic aldehyde. There was not sufficient material to identify the other peaks, but on the

basis of elution volume, peak II is probably laminaribiosyl-glycerol. The baker's yeast G_1 preparation (47·0 mg) was also fragmented on Smith degradation and yielded 25·0 mg of resistant glucan.

The insoluble polysaccharide obtained after Smith degradation was subjected to methylation analysis (Table 2). As expected, this material was entirely (1→3)-linked and contained no (1→6)-linked residues. However, a small percentage of the residues were still tri-substituted at C-1, C-3 and C-6 as in the undegraded preparations. This implies the presence of structural unit (A) in the original molecule as opposed to the unit (B): the latter would have given rise to a more linear molecule on Smith degradation.

Buffer extraction of cell walls

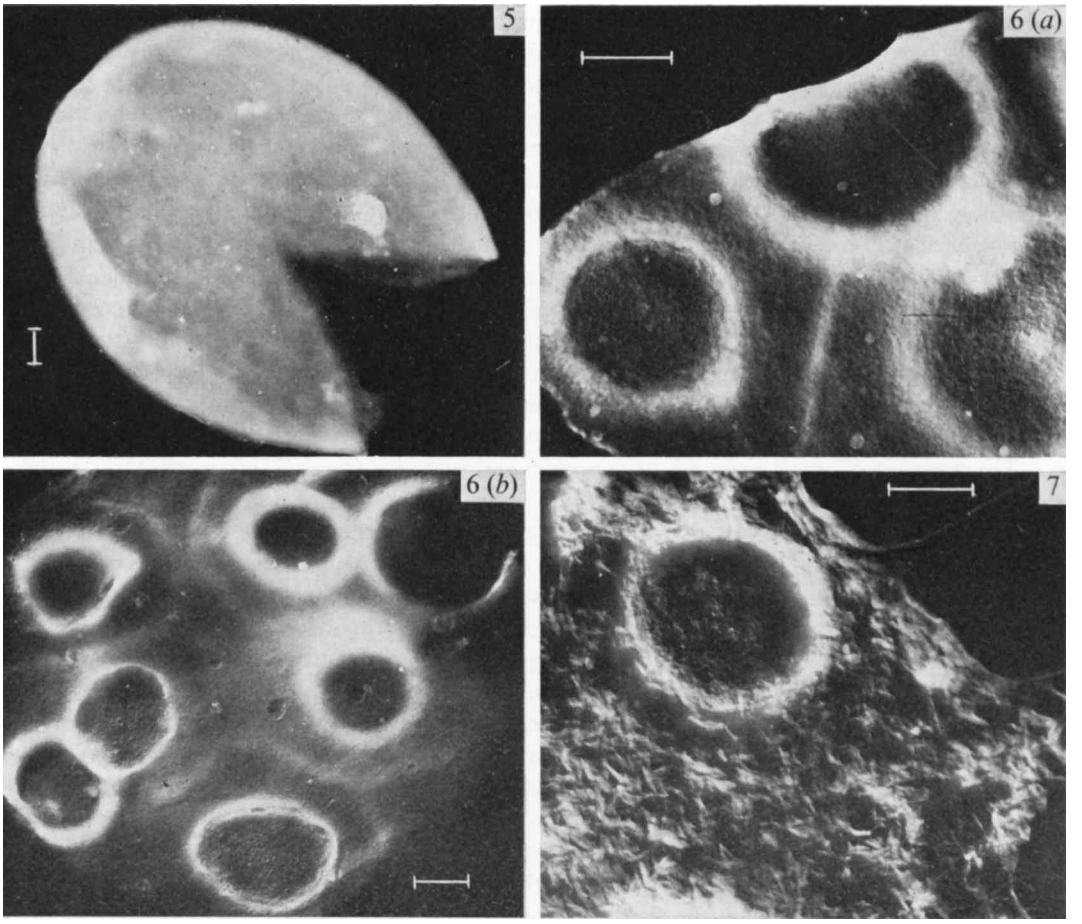
Cell-wall mannan is routinely extracted by autoclaving yeast cells with sodium citrate buffer, pH 7·0 (Phaff, 1971). Extraction of isolated cell walls of *S. cerevisiae* 1109 according to this procedure gave rise to two distinct insoluble layers on centrifuging. The supernatant solution gave a precipitate with Fehling's solution and was presumed to contain mannan. The first of the insoluble layers (B_1) was loosely packed and was readily separated from the more firmly sedimented layer (B_2). The latter retained the original cell-wall shape and was not studied further. B_1 was washed five times with distilled water, then briefly examined. It was soluble in M-sodium hydroxide and on acid hydrolysis gave mostly glucose. Incubation with an *endo*-(1→3)-β-D- or an *endo*-(1→6)-β-D-glucanase (Fleet & Phaff, 1974*a*) released substantial quantities of reducing sugars indicating the presence of (1→3)-β-D- and (1→6)-β-D-glucosidic linkages. Fraction B_1 was therefore considered to be very similar to the G_1 preparation described in this study.

Electron microscopy

The external surface of *S. cerevisiae* cell walls was very smooth (Fig. 5), and bud scars were not generally apparent. After the alkali extraction (Fig. 1), the cell walls exhibited a somewhat deformed original shape (Fig. 6*a*, *b*). The wall surface now had a slightly granular appearance and bud scars were very conspicuous and numerous. Some cell walls were virtually obliterated by the presence of these features. However, the extracted walls did not show any fibrillar structure. As a control on the techniques used, baker's yeast cell walls were examined after boiling with 2 % hydrochloric acid for 2 h. The procedure produces the fibrillar 'hydroglucan' shown in Fig. 7, as originally described by Houwink & Kreger (1953).

DISCUSSION

The observation by Eddy & Woodhead (1968) that the cell walls of *Saccharomyces carlsbergensis* contain approximately 20 % of an alkali-soluble glucan has been confirmed for *S. cerevisiae* by our studies. This glucan appears, at first sight, to be distinct from the two other cell-wall glucan components recently described by Manners *et al.* (1973*a*, *b*, 1974). Hence, it is probable that the yeast cell wall contains at least three separate glucan species.



All bar markers represent 1 μm.

Fig. 5. Untreated cell wall of *S. cerevisiae* 1109.

Fig. 6. Alkali-extracted cell wall (see Fig. 1) of (*a*) *S. cerevisiae* 1109; (*b*) baker's yeast.

Fig. 7. Hydroglucan from baker's yeast cell walls (see Results).

It should be emphasized that in this study, the alkali-soluble glucan was obtained from cell-wall preparations, whilst in the previous studies, intact yeast cells were used as starting material. Bacon *et al.* (1969) have shown that glucans are more readily extracted by alkali from isolated cell walls, than from intact cells. The reasons for this difference, which Bacon *et al.* (1969) have discussed in detail, are not clearly understood. Thus caution is required in comparing and interpreting the results of the extraction with alkali of intact cells and of wall preparations.

Under the culture conditions used, the cell walls of *S. cerevisiae* contained approximately 30 % mannan components (S_1, S_2 and a remaining 9 % in wall residue) and 60 % glucan components (G_1, G_2 and wall residue). In previous studies on the composition of baker's yeast cell walls, values of 30 to 35 % each were reported for the content of the mannan and glucan components (Northcote & Horne, 1952; Roelofsen, 1953; cf. Bacon *et al.*, 1969). In these cases, cell walls were extracted with hot alkali and consequently incurred the risk

of degrading the alkali-soluble glucan component (Eddy & Woodhead, 1968; Bowden & Hodgson, 1970). It is not surprising, therefore, that low values for the glucan content of the walls were obtained. Mill (1966) has reported values around 45 % for both the glucan and mannan wall components of *S. cerevisiae* and these are possibly more reliable figures since they are based on the determination of glucose and mannose contents in cell-wall hydrolysates rather than in extracted fractions. However, any study of the composition of yeast cell walls is primarily dependent on the quality of the starting cell-wall material. It is now known that yeast cell walls contain associated (1→3)-β-D-glucanase activity which, if not checked, causes very substantial wall hydrolysis immediately after cell disruption (Fleet & Phaff, 1974*b*). The effect of such activity is to reduce the overall glucan content. The cell walls used in our study were prepared under conditions whereby associated glucanases were inactive and this may explain their much higher glucan content. As demonstrated by McMurrough & Rose (1967), cell-wall composition is also determined by the environment in which the yeast cells are grown. Environments of glucose abundance, such as used in our studies, are more favourable to a higher glucan content.

The presence of small amounts of mannose and nitrogen (presumably as protein) in the alkali-soluble glucan is not unexpected and suggests that, in the native wall, this glucan fraction may have been part of a much larger polysaccharide–protein complex. Glucan–mannan–protein complexes have previously been isolated from baker's yeast cell walls. Kessler & Nickerson (1959) obtained two such complexes on extracting cell walls with M-potassium hydroxide for 1 h at 30 °C under nitrogen, whilst Korn & Northcote (1960) obtained two similar complexes with a milder extraction procedure using ethylenediamine. In both cases, one of the complexes was insoluble at neutral pH values and it is possible that the glucan moiety of this macromolecule is similar in structure to the glucan described in our study. Although there has been some progress in elucidating the nature of the mannan–protein linkage (Ballou, 1974), the nature of any glucan–protein or glucan–mannan linkages is as yet unresolved.

Chemical analysis of the alkali-soluble glucan has shown that it contains mostly (1→3)-β-D linkages. In this respect and in size (DP about 1500), it is very similar to the alkali-insoluble glucan which forms the rigid component of *S. cerevisiae* cell walls (Manners *et al.*, 1973*a*). Unlike the latter, however, the alkali-soluble glucan contains a significant percentage (8 to 12 %) of residues linked solely by C-1 and C-6. Both glucans show approximately 3 to 4 % branching involving C-1, C-3 and C-6. The presence of these 150 or so (1→6)-linked residues seems to be sufficient to alter the solubility properties of the molecule. Consequently, the arrangement of these linkages is important but the exact details cannot be deduced from the available evidence. However, certain facts can be stated. Since gentiotetrose was present in partial acid hydrolysates of the glucan, sequences of at least three (1→6)-linked glucose residues must occur. Some of these (1→6) linkages are dispersed among longer sequences of (1→3) linkages since the molecule was fragmented to a limited extent by Smith degradation. Since glucosyl-glycerol and, most likely, laminaribiosyl-glycerol were products of Smith degradation, some of the occasional laminaribiosyl and laminaritriosyl units may be flanked on either side by (1→6) linkages. Finally, some of the 3 to 4 % of (1→6) branch points do not connect directly with other (1→6)-linked residues because of the presence of structure (A) (see Results) in the Smith-degraded glucan (Table 2). Further studies are in progress to determine the finer structural details of this new glucan.

Alkali-soluble glucans occur in other yeasts (Phaff, 1971), but in cases where they have been studied, such as in Cryptococcus species (Bacon *et al.*, 1968) and Schizosaccharomyces species (Bush *et al.*, 1974), the molecules have been predominantly α-(1→3)-linked. An

alkali-soluble glucan similar in composition to the one described in this study was recently found in the hyphal walls of the fungus *Pythium acanthicum* (Sietsma *et al.*, 1975).

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