The Enzymic Degradation of an Alkali-soluble Glucan from the Cell Walls of *Saccharomyces cerevisiae*

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SUMMARY

An alkali-soluble glucan from the cell walls of *Saccharomyces cerevisiae* NCYC1109 has been hydrolysed with a purified endo-(1→3)- β -D-glucanase and an endo-(1→6)- β -D-glucanase from *Bacillus circulans* WL-12. The products of enzyme action include various oligosaccharide and polysaccharide fractions which have been separated by gel filtration and characterized, giving new information on the fine structure of the glucan. The isolated cell walls have also been subjected to enzymic hydrolysis. The results suggest that part of the cell-wall mannan is held in place by a glucan component.

INTRODUCTION

In a previous paper (Fleet & Manners, 1976), we described the isolation, composition and general structure of an alkali-soluble glucan component from the cell walls of *Saccharomyces cerevisiae*. This new wall glucan contained both (1→3)- β and (1→6)- β linkages. The determination of polysaccharide fine structure is greatly facilitated by the judicious use of specific hydrolytic enzymes. Fleet & Phaff (1974a) reported the purification and properties of an endo-(1→3)- β -D-glucanase and an endo-(1→6)- β -D-glucanase from *Bacillus circulans* WL-12 suitable for such application. The present paper describes the use of these two enzymes in determining some finer structural features of the alkali-soluble glucan. In addition, the action of these two enzymes on the glucan is compared with their action on intact yeast cell walls. A preliminary account of part of these results has been given (Fleet & Manners, 1975).

METHODS

Cell walls and glucan fraction. The isolation of cell walls from *S. cerevisiae* NCYC1109 and from baker's yeast and the preparation of the alkali-soluble glucan fraction G₁ from these walls have been described previously (Fleet & Manners, 1976).

Analytical procedures. Methods for total and partial acid hydrolysis, glucose and carbohydrate estimation, methylation analysis and paper chromatography were as described by Fleet & Manners (1976). Protein was determined by the method of Lowry *et al.* (1951). Gel filtration of polysaccharide hydrolysates was done either on a column (115 × 1.5 cm) of Bio-Gel P-2 (BioRad) or on a column (85 × 1.5 cm) of Sepharose 6B (Pharmacia). The columns were equilibrated and eluted with 0.05 M-NaCl. Fractions of 3.0 ml were generally

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Table 1. *The products of glucanase action on G₁ preparations and yeast (1→6)-β-D-glucan*

After enzyme action for 4 h, products were identified by paper chromatography using solvent A (Fleet & Manners, 1976). The + values refer to the relative spot intensities on the chromatograms. The figures in parentheses are mobility values relative to glucose (1.0).

(1→3)-β-D-Glucanase action on G ₁ *	(1→6)-β-D-Glucanase action on G ₁ *	(1→6)-β-D-Glucanase action on yeast (1→6)-β-D-glucan†
Glucose, 10+	Glucose, 4+	Glucose, 6+
Laminaribiose, 10+ (0.76)	Laminaribiose, 1+ (0.76)	Gentiobiose, 10+
Laminaritriose, 10+ (0.51)	Gentiobiose, 10+ (0.44)	3 ² -β-Glucosylgentiobiose, 6+
Laminaritetraose, 4+ (0.35)	Laminaritriose, 1+ (0.51)	Gentiotriose, 10+
Laminaripentaose, 4+ (0.24)	3 ² -β-Glucosylgentiobiose, 4+ (0.31)	Mixed-linkage tetrasaccharide, 10+
Gentiotriose, 4+ (0.18)	Gentiotriose, 4+ (0.18)	Gentiotetraose, 10+
	Mixed-linkage tetrasaccharide, 4+ (0.12)	Gentiopentaose, 10+
	Gentiotetraose, 4+ (0.08)	

* G₁ preparations from both *S. cerevisiae* NCYC1109 and baker's yeast gave the same results.

† Preparation obtained from baker's yeast by Manners *et al.* (1973*b*).

collected and their carbohydrate content was measured by the phenol-sulphuric acid assay (Dubois *et al.*, 1956).

Glucanase preparations. The (1→3)-β- and (1→6)-β-D-glucanases were produced by *Bacillus circulans* WL-12 grown in a salts medium with baker's yeast cell walls as the carbon source. The two enzymes were extensively purified as described by Fleet & Phaff (1974*a*). After purification, the enzymes were checked for hydrolytic action and substrate specificity. The (1→3)-β-D-glucanase was specific for (1→3)-β-D-linked glucans (laminarin) and hydrolysed these molecules in a random manner, i.e. it was an endo-enzyme. The (1→3)-β-D-glucanase did not hydrolyse the (1→6)-β-D-glucans, pustulan (Hellerqvist, Lindberg & Samuelsson, 1968), or luteose (Nakamura & Tanabe, 1963). Similarly, the (1→6)-β-D-glucanase (which was also an endo-enzyme) was specific for the (1→6)-β-D-glucosidic linkage and did not release reducing sugars from laminarin. These results are consistent with those previously published (Fleet & Phaff, 1974*a*).

Enzymic hydrolysis of G₁ preparations and cell walls. Before use, cell walls were first heated in water at 100 °C for 5 min to inactivate endogenous glucanase activities (Fleet & Phaff, 1974*b*). Cell walls or G₁ preparations were uniformly suspended in 0.25 M-sodium succinate buffer pH 5.0 using a Potter homogenizer. Enzyme solution was then added so that, in all cases, the final concentration of the cell walls or G₁ was 2 mg ml⁻¹ and of the enzyme was 0.5 unit ml⁻¹. All suspensions were made 0.01 % (w/v) with respect to sodium azide as a precaution against microbial contamination. Suspensions were incubated at 30 °C with slow rotation. When required, samples were removed for the determination of reducing sugar or total carbohydrate solubilization.

RESULTS

Action of (1→3)-β- and (1→6)-β-D-glucanases on fraction G₁

The (1→3)-β-D-glucanase caused immediate and extensive hydrolysis of both G₁ preparations (Figs 1 and 6). Approximately 25 % of the initial glucan material remained insoluble under the conditions of hydrolysis but was readily solubilized on heating. However, this material precipitated again on cooling. The soluble products after hydrolysis for 1 to

Fig. 1. The action of the (1→3)-β-D-glucanase (—) and the (1→6)-β-D-glucanase (---) on yeast cell-wall glucan preparations: (a) G₁ from *S. cerevisiae* NCYC1109; (b) G₁ from baker's yeast; (c) yeast (1→6)-β-D-glucan; (d) yeast alkali-insoluble (1→3)-β-D-glucan. ●, Release of reducing sugars; ○, total solubilization of carbohydrate.

4 h are listed in Table 1. Similar products were noted after 50 h hydrolysis, but the proportions of laminaritetraose and laminaripentaose were much lower and gentiotriose had increased slightly. Gentiotriose was not formed. The presence of gentiotriose was confirmed by the fact that it was hydrolysed by the (1→6)-β-D-glucanase to give glucose and gentiobiose.

The G₁ preparations were also hydrolysed by the (1→6)-β-D-glucanase, although to a much lesser extent than by the (1→3)-β-D-glucanase (Fig. 1 *a, b*). The soluble products of this reaction are also shown in Table 1. The proportions of gentiotriose and gentiotetraose were much lower after hydrolysis for 50 h and the amounts of the two mixed-linkage oligosaccharides (R_{glo} 0.31 and R_{glo} 0.12) had substantially increased. The presence of laminarisaccharides and mixed-linkage oligosaccharides in the products suggests that some of the (1→3)-β-D-linked residues are flanked by certain sequences of (1→6)-β-D-linked residues.

These enzymic studies confirm the chemical observations (Fleet & Manners, 1976) that the G₁ wall fraction is a predominantly (1→3)-β-D-linked glucan containing a small percentage of (1→6)-β-D-linked residues. The latter can exist in blocks of at least three units since gentiotetraose was a product of hydrolysis.

For comparison, Fig. 1 (*c, d*) shows the action of the two glucanases on yeast alkali-insoluble (1→3)-β-D-glucan (Manners, Masson & Patterson, 1973*a*) and yeast (1→6)-β-D-glucan (Manners *et al.*, 1973*b*). The alkali-insoluble (1→3)-β-D-glucan was only weakly cleaved by the (1→3)-β-D-glucanase (giving traces of laminarisaccharides) and was not hydrolysed at all by the (1→6)-β-D-glucanase. The yeast (1→6)-β-D-glucan was not hydrolysed by the (1→3)-β-D-glucanase but was extensively degraded by the (1→6)-β-D-glucanase yielding the products shown in Table 1. Large amounts of the mixed-linkage oligosaccharides had accumulated after 50 h. The trisaccharide (R_{glo} 0.31) was therefore isolated by preparative paper chromatography and identified. Total acid hydrolysis yielded only

Fig. 2. Bio-Gel P-2 chromatography of (1→3)- β -D-glucanase hydrolysates of (a) G_1 from *S. cerevisiae* NCYC1109, (b) G_{1-6} from *S. cerevisiae* NCYC1109, (c) cell walls from *S. cerevisiae* NCYC1109.

glucose; on partial acid hydrolysis, gentiobiose and laminaribiose were formed in addition to glucose. Reduction of the trisaccharide with potassium borohydride, followed by partial acid hydrolysis, yielded glucose and laminaribiose as reducing sugars and sorbitol and gentiobi-itol as non-reducing sugars (Peat, Whelan & Edwards, 1958). Incubation of the trisaccharide with an exo-(1→3)- β -D-glucanase (kindly supplied by Dr G. Wilson) produced glucose and gentiobiose. Collectively these results characterize the oligosaccharide as 3²- β -glucosylgentiobiose, as opposed to 6²- β -glucosyllaminaribiose or 3,6-di- β -glucosylglucose: $G^1\text{---}^3G^1\text{---}^6G$.

Column chromatography of the G_1 enzyme hydrolysates

The total carbohydrate release was far higher than the corresponding reducing sugar value (Fig. 1*a, b*). This suggested that a proportion of the solubilized products were large molecules. The solubilized material was therefore fractionated by gel filtration.

(1→3)- β -D-glucanase hydrolysates. G_1 (100 mg) from *S. cerevisiae* NCYC1109 was hydrolysed with (1→3)- β -D-glucanase for 50 h as described in Methods. Enzymic activity was destroyed by heating the reaction mixture at 100 °C for 5 min. After cooling, the insoluble component was separated by centrifugation and accounted for 31 % of the initial polysaccharide material. This residue will be referred to as G_{1-3} (see Fig. 6). The supernatant fraction was chromatographed on Bio-Gel P-2 (Fig. 2*a*). Two major peaks (A and B) and two minor peaks (C and D) of carbohydrate were found. Peak A (15.2 mg) eluted with the void volume and was polysaccharide in nature. Peaks B (24.6 mg), C (6.3 mg) and D (7.1 mg) were identified as laminaritriose, laminaribiose and glucose respectively.

On total acid hydrolysis, samples of peak A gave glucose (87 %) and mannose (13 %). On partial acid hydrolysis, glucose, gentiobiose, gentiotriose and gentiotetraose were the major products together with smaller amounts of laminaribiose, laminaritriose and mannose. As expected, peak A was not hydrolysed by the endo-(1→3)- β -D-glucanase but was degraded by the endo-(1→6)- β -D-glucanase to give mainly glucose, gentiobiose, 3²- β -glucosyl-gentiobiose and the mixed-linkage tetrasaccharide (R_{glc} 0.12). Traces of gentiotriose and mannose were also formed.

Peak A was subjected to further chromatographic examination in an attempt to determine its homogeneity. Samples of the material were excluded from Bio-Gel P-30 and P-150 columns. Fractionation of peak A on Sepharose 6B chromatography (Fig. 3a) gave two incompletely resolved peaks, E and F. Because of the considerable overlap, only the groups of fractions indicated (Fig. 3a) were combined for further examination. These are designated as groups 1 and 2. On total acid hydrolysis, samples from group 1 yielded 75 % glucose and 25 % mannose. Partial acid hydrolysis yielded predominantly glucose and gentiosaccharides together with smaller amounts of mannose. (1→3)- β -D-linked oligosaccharides or mannosaccharides were absent. It appears, therefore, that the first peak eluted from the Sepharose 6B column was primarily a (1→6)- β -D-linked glucan. It is not clear from these results whether mannose is covalently linked to this glucan.

Similar analysis of group 2 fractions (Fig. 3a) gave 85 % glucose and 15 % mannose. It is possible that this small percentage of mannose was a contamination from the preceding peak. Partial acid hydrolysates contained predominantly glucose and gentiobiose but no higher gentiosaccharides. Smaller amounts of the mixed-linkage oligosaccharides (Table 1; R_{glc} 0.31 and 0.12) were also formed. These products are consistent with a sequence consisting of alternating (1→3)- β and (1→6)- β -D linkages. The absence of laminaribiose in the hydrolysates is consistent with the lability of this linkage when adjacent to a (1→6)- β -bond. Such a structure would also explain the presence of glucosylglycerol in the products of Smith degradation of the original glucan (Fleet & Manners, 1976).

(1→6)- β -D-glucanase hydrolysates. G_1 (100 mg) was hydrolysed with the endo-(1→6)- β -D-glucanase for 50 h. The insoluble undegraded material was isolated by centrifugation and will be referred to as G_{1-6} (yield 83.4 mg; see Fig. 6). The soluble hydrolysis products were concentrated and fractionated by chromatography on Bio-Gel P-2 (Fig. 4). Five main peaks (G, H, J, K, L) of carbohydrate were resolved. Peaks H, J, K and L were identified respectively as gentiotetraose and mixed-linkage tetrasaccharide (R_{glc} 0.12); gentiotriose and 3²- β -glucosylgentiobiose; gentiobiose; glucose. Peak G, which accounted for 1.85 % of the original material, eluted with the void volume and samples of this fraction were also excluded from Bio-Gel P-30 and P-150 columns. Peak G was considered to be polysaccharide in nature and on acid hydrolysis gave mostly mannose with smaller amounts of glucose. Yields of this material were insufficient for further detailed examination.

Characterization of G_{1-3} and G_{1-6}

As described above, these are the insoluble residues left after either (1→3)- β - or (1→6)- β -D-glucanase hydrolysis of G_1 preparations.

G_{1-3} . This material was not hydrolysed further by the endo-(1→3)- β -D-glucanase; in addition, it was not hydrolysed by the endo-(1→6)- β -D-glucanase. Total acid hydrolysis gave only glucose. On partial acid hydrolysis, the following products were obtained: glucose 10+, laminaribiose 4+, laminaritriose 3+, gentiobiose 3+, laminaritetraose 2+, laminaripentaose 2+, gentiotriose 2+, and higher unresolved oligosaccharides. As

Fig. 3

Fig. 4

Fig. 3. Sepharose 6B chromatography of (a) peak A (Fig. 2a), (b) peak M (Fig. 2c), (c) peak T (Fig. 4b).

Fig. 4. Bio-Gel P-2 chromatography of (1 $\rightarrow$ 6)- β -D-glucanase hydrolysates of (a) G_1 from *S. cerevisiae* NCYC1109, (b) cell walls from *S. cerevisiae* NCYC1109.

expected, methylation analysis (Table 2) showed a predominantly (1 $\rightarrow$ 3)- β -D-linked glucan with small percentages of branching and (1 $\rightarrow$ 6)- β -D-linked residues. The degree of branching and proportion of (1 $\rightarrow$ 6)- β -D-linked residues were smaller than in the parent G_1 molecule (see Table 2). The degraded molecule had a degree of polymerization (DP) of 270 (cf. 1330 for undegraded G_1 preparation) as determined by the sorbitol dehydrogenase method.

G_{1-6} . This material was not further hydrolysed by the (1 $\rightarrow$ 6)- β -D-glucanase. However, it was extensively degraded by the (1 $\rightarrow$ 3)- β -D-glucanase yielding an insoluble residue, as described previously for the hydrolysis of G_1 by this enzyme. Bio-Gel P-2 chromatography of the solubilized components gave the elution profile shown in Fig. 2(b). The products were similar to those described for Fig. 2(a) except that the excluded peak was much smaller. This is consistent with the evidence that the excluded peak was essentially a (1 $\rightarrow$ 6)- β -D-linked component. Methylation analysis of G_{1-6} (Table 2) again showed a predominantly (1 $\rightarrow$ 3)- β -D-linked glucan. A small percentage (about 1%) of (1 $\rightarrow$ 6)- β -D-linkages were resistant to enzymic hydrolysis. These probably occur as isolated di- or tri-saccharide units in larger blocks of (1 $\rightarrow$ 3)- β -D-linked residues. This is consistent with the previous Smith degradation studies when fragmentation of G_1 was found (Fleet & Manners, 1976). G_{1-6} was very resistant to acid hydrolysis and so the DP could not be measured by the sorbitol dehydrogenase method.

Table 2. Methylation analysis of G_1 preparations after degradation by $(1 \rightarrow 3)$ - β - and $(1 \rightarrow 6)$ - β -D-glucanases

O-Methyl-D-glucitol acetate derivative	Type of linkage	Composition (mol %)		
		G_{1-3}	G_{1-6}	G_1^*
2,3,4,6-Tetra-	Non-reducing end groups	3.2	2.9	3.7
2,4,6-Tri-	$(1 \rightarrow 3)$ -	93.8	93.8	84.7
2,3,4-Tri-	$(1 \rightarrow 6)$ -	1.5	1.0	8.3
2,4-Di-	Branch points at C-1, C-3 and C-6	1.5	2.3	3.3

* Data from Fleet & Mannes (1976).

Fig. 5. The action of the $(1 \rightarrow 3)$ - β -D-glucanase (a) and the $(1 \rightarrow 6)$ - β -D-glucanase (b) on isolated cell walls of *S. cerevisiae* NCYC1109. The broken lines show the action of a mixture of the two enzymes. ●, Release of reducing groups; ○, total solubilization of carbohydrate.*Action of the $(1 \rightarrow 3)$ - β - and $(1 \rightarrow 6)$ - β -D-glucanases on cell walls of *S. cerevisiae* NCYC1109*

Cell walls were hydrolysed with either the $(1 \rightarrow 3)$ - β - or $(1 \rightarrow 6)$ - β -D-glucanase or with a combination of the two enzymes (Figs 5 and 6). Very little degradation was apparent with either enzyme system when hydrolysis was monitored only by the release of reducing groups. However, total carbohydrate solubilization was much higher (up to one third of the initial wall material after 50 h) suggesting that mostly large molecules were being released during enzymic degradation. The monosaccharide composition of the solubilized carbohydrate, examined by total acid hydrolysis, was 70 % glucose and 30 % mannose for the $(1 \rightarrow 3)$ - β -D-glucanase hydrolysate and 60 % glucose and 40 % mannose for the $(1 \rightarrow 6)$ - β -D-glucanase hydrolysate.

Nature of the solubilized products

Examination by paper chromatography indicated that these were the same as those obtained from the corresponding hydrolyses of the alkali-soluble G_1 preparations from the cell wall (Table 1 and Fig. 6). To identify the higher molecular weight species, large-scale enzymic digestions (300 mg cell walls) were prepared. After hydrolysis for 56 h, the remaining insoluble material was removed by centrifugation and the supernatant was concentrated and fractionated by gel chromatography.

Fig. 6. Flow diagrams for the analysis of G₁ and cell-wall enzyme hydrolysates.

(1→3)- β -D-glucanase hydrolysate. The elution profile on Bio-Gel P-2 chromatography is shown in Fig. 2(c). Most of the carbohydrate solubilized from the cell walls was excluded from the column (peak M) and was polysaccharide in nature (cf. Fig. 2a). Peaks N, O and P were identified as laminaritrise, laminaribiose and glucose respectively. Total acid hydrolysis of peak M fractions liberated 36 % glucose and 64 % mannose. Peak M was fractionated further by Sepharose 6B chromatography and the results are presented in Fig. 3(b) so that they can be compared with the fractionation of the G_1 (1→3)- β -D-glucanase hydrolysate (Fig. 3a). Three overlapping peaks Q, R and S were obtained and representative fractions (indicated in Fig. 3b) were combined and examined. The contents of glucose and mannose in total acid hydrolysates of each group of fractions were: group 1, 16 % glucose, 84 % mannose; group 2, 7 % glucose, 93 % mannose; group 3, 50 % glucose, 50 % mannose. Because of its high glucan content, a sample of group 3 was incubated with the (1→6)- β -D-glucanase. Gentiosaccharides were released in substantial quantities suggesting that the glucan component of this polysaccharide fraction was predominantly (1→6)- β -D-linked. This polysaccharide peak, therefore, is similar in composition to peak E of Fig. 3(a); both peaks eluted in the same position.

(1→6)- β -D-glucanase hydrolysate. The elution profile on Bio-Gel P-2 chromatography of this digest is presented in Fig. 4(b). Most of the solubilized polysaccharide was excluded from the column (peak T). Peaks U, V, W and X were identified as the same as the corresponding peaks in Fig. 4(a). Acid hydrolysis of peak T gave 3 % glucose and 97 % mannose. Peak T gave rise to a disperse peak on Sepharose 6B chromatography (Fig. 3c). Hydrolysis of the fractions indicated in Fig. 3(c) liberated 2 % glucose and 98 % mannose. This polysaccharide peak is probably similar to the corresponding peak in Fig. 3(b).

Protein release from cell walls on glucanase hydrolysis

The protein solubilized by glucanase action was determined using the material excluded from the Bio-Gel P-2 columns. Allowances were made for the added glucanase protein which was also excluded from these columns. However, the action of the glucanase caused the solubilization of only trace amounts of cell-wall protein. From 300 mg cell-wall material, the (1→3)- β -D-glucanase solubilized only 250 μ g protein while the (1→6)- β -D-glucanase gave 650 μ g.

Cell-wall composition after glucanase hydrolysis

Glucose was the main monosaccharide component of the insoluble cell-wall material left after glucanase hydrolysis. However, these wall residues (from either enzyme treatment) still contained 15 to 20 % mannose, indicating that the cell-wall mannan was not completely removed by the action of the glucanases.

DISCUSSION

Structure of the alkali-soluble glucan

In our previous paper (Fleet & Manners, 1976), the alkali-soluble glucan from the cell walls of *S. cerevisiae* NCYC1109 was characterized as a molecule containing approximately 1330 glucose residues, with 85 % (1→3)- β -D linkages, 8 % (1→6)- β -D linkages and 3 % of residues triply-linked through C-1, C-3 and C-6. The glucan also contained 1 to 2 % mannose residues. Any interpretation of the fine structure of this macromolecule, however, is limited by the assumptions which must be made about its purity. In the absence of any evidence for heterogeneity for this glucan (Fleet & Manners, 1976), it will be assumed that both the (1→3)- β -D linkages and the (1→6)- β -D linkages are present in the same molecule.

Similarly, it will be assumed that the small amount of mannose present is not due to contamination by the cell-wall mannan component (Phaff, 1971) although this possibility cannot be entirely eliminated at this stage.

The alkali-soluble glucan was extensively degraded by the (1→3)- β -D-glucanase with the liberation of laminarisaccharides, glucose and small amounts of gentiotriose. Hence the molecule contained a substantial proportion of (1→3)- β -D-linked residues. The enzyme also released a soluble polysaccharide fraction (peak A, Fig. 2) which was heterogeneous (Fig. 3a). The major component of this polysaccharide fraction (peak E, Fig. 3a) contained (1→6)- β -D-linked residues since gentiosaccharides were liberated both on partial acid hydrolysis and on enzymic digestion with a (1→6)- β -D-glucanase. Mannose, which accounted for virtually all of the mannose in the original glucan, was also found in total acid hydrolysates of this peak. Whether peak E is a covalently-linked glucomannan complex is not clear from the data, since glucose-mannose oligosaccharides were not detected in partial acid hydrolysates. However, in view of the small amounts of mannose present in the original glucan molecule (15 to 25 residues), such oligosaccharides could have been overlooked.

The action of the (1→3)- β -D-glucanase also yielded a resistant fraction G_{1-3} in 25 to 30 % yield. This fraction which had an apparent DP of 270 contained 94 % (1→3)-linked, 1.5 % (1→6)-linked and 1.5 % triply-linked glucose residues. Thus, four branch points were present in this fragment. Some of the (1→6)-linked residues were adjacent, since gentiobiose and gentiotriose were found in partial acid hydrolysates. Despite the very high proportion of (1→3) linkages, G_{1-3} was not further hydrolysed by the (1→3)-glucanase. This observation could relate to the physical state of the molecule (see Rees, 1973). The alkali-insoluble (1→3)- β -D-linked glucan of baker's yeast cell walls (Manners *et al.*, 1973a) was also largely resistant to the action of this particular glucanase (Fig. 1d). The resistant G_{1-3} fraction may represent the 'core' of the alkali-soluble glucan.

The action of the (1→6)- β -D-glucanase on the alkali-soluble glucan was much less than the (1→3)- β -D-glucanase (Fig. 1), as expected from the ratio of the two types of linkages present in the molecule. The soluble products of hydrolysis were gentio- and mixed-linkage oligosaccharides and a soluble polysaccharide fraction (peak G, Fig. 4), which produced predominantly mannose with minor amounts of glucose on total acid hydrolysis. Thus, hydrolysis of the alkali-soluble glucan with the (1→6)- β -D-glucanase releases and solubilizes the associated mannan material. This observation combined with the finding of a possible (1→6)- β -D-glucan-mannan complex (peak E, Fig. 3) suggests that some (1→6)-linked D-glucose residues are involved in the association of a mannan fragment with the predominantly (1→3)- β -D-linked glucan.

The major product of (1→6)- β -D-glucanase action on the glucan was the resistant fraction G_{1-6} (yield 83 %). This material was shown by methylation analysis (Table 2) to be similar in composition to G_{1-3} . The small percentage of (1→6)-linked residues which survived the (1→6)- β -D-glucanase hydrolysis, and remained in G_{1-6} are probably dispersed as occasional di- and trisaccharide units among larger blocks of (1→3)-linked residues. This would be consistent with the observed fragmentation pattern of the glucan on Smith degradation (Fleet & Manners, 1976).

The overall structure of the alkali-soluble glucan that emerges is of a macromolecule with a (1→3)- β -D-glucan type 'core' having a low degree of branching (about 2.0 %). The 'core' also contains occasional (1→6)-linked residues. To this 'core' are attached mainly (1→3)- β -D-linked side chains which may contain secondary branching, also in the form of (1→3)-linked side chains. This would represent the structure remaining after the

(1→6)- β -D-glucanase hydrolysis. The (1→3)- β -D-linked side chains represent the proportion of the molecule susceptible to (1→3)- β -D-glucanase cleavage. Of the 1330 residues present in the original glucan, about 40 (3 %) are branch points. Side chains containing either mainly (1→6)-linked residues or a mixture of (1→6)-linked and (1→3)-linked residues (peaks E and F, Fig. 3) are also present in the molecule and these may be attached to the (1→3)-linked side chains described above since they are released by (1→3)- β -D-glucanase hydrolysis. However, as there are only about 100 residues in the glucan (8 %) which are linked solely C-1-C-6, the number of such side chains would be very few indeed.

The alkali-soluble glucan as isolated always contains a fragment of the cell-wall mannan component which can be released by hydrolysis with the (1→6)- β -D-glucanase. If this fragment is covalently linked to the glucan, it would appear from our results that (1→6)-linked β -D-glucose residues are involved in this function. Glucomannan complexes obtained by the alkali-extraction of cell walls of *S. cerevisiae* have been described previously (Kessler & Nickerson, 1959) but their structures were not reported.

*Action of the Bacillus circulans WL-12 glucanases on the cell walls
of S. cerevisiae NCYC1109*

In a previous paper (Kopecká, Phaff & Fleet, 1974), the actions of the endo-(1→3)- β -D-glucanase and the endo-(1→6)- β -D-glucanase on isolated cell walls of *S. cerevisiae* were studied using the electron microscope. These enzymes, acting singly or in combination, brought about the removal of an amorphous surface layer from the cell walls to reveal bud scars and a fibrillar glucan network. The present investigation has focused on the chemical changes of this reaction. Although the two enzymes did not produce any visible lysis of *S. cerevisiae* cell walls (cf. Tanaka & Phaff, 1965; Fleet & Phaff, 1974a), they nevertheless caused 30 to 40 % solubilization of the cell-wall polysaccharide material. The products of hydrolysis from the action of either enzyme were a mixture of polysaccharide and oligosaccharide components. The nature of the oligosaccharide products clearly indicated that (1→3)- and (1→6)- β -D-glucosidic linkages within the cell wall had been cleaved. The following discussion will show that these linkages probably formed part of the alkali-soluble glucan fraction of the wall.

The (1→3)- β -D-glucanase was more effective at hydrolysing the cell wall than the (1→6)- β -D-glucanase and this is consistent with the action of these enzymes on the alkali-soluble glucan (Figs 1 and 5). The glucose-containing products of cell-wall hydrolysis by the (1→3)- β -D-glucanase accounted for about 20 % of the cell-wall material, which corresponds approximately with the wall content of alkali-soluble glucan (Eddy & Woodhead, 1968; Fleet & Manners, 1976). The oligosaccharide products of cell-wall hydrolysis from either enzyme were identical to those obtained by the action of these enzymes on the isolated alkali-soluble glucan (Table 1), strongly suggesting that this glucan is the site of enzyme action in intact cell walls.

The polysaccharide products of (1→3)- β -D-glucanase action on the walls were heterogeneous and included some predominantly mannan-containing components (Fig. 3b). One of these components, however, (peak S, Fig. 3b) contained equal amounts of glucan and mannan and the glucan fraction contained a high proportion of (1→6)- β -D-linkages. This association of (1→6)- β -D-glucan and mannan is similar to that found in peak E (Fig. 3a) which was obtained by the hydrolysis of isolated alkali-soluble glucan with (1→3)- β -D-glucanase. The polysaccharide products of (1→6)- β -D-glucanase action on the cell walls were mostly mannan-containing components, but, as expected, the glucan-mannan component (peak S, Fig. 3b) was absent since the glucan moiety would have been degraded.

Bacon *et al.* (1969) have previously described the solubilization of cell-wall mannan material by the action of an endo-(1→6)- β -D-glucanase. Collectively, these separate observations suggest an association of the cell-wall mannan with some (1→6)-linked β -D-glucose residues.

The heterogeneity of the mannan components obtained on gel filtration of the wall hydrolysates coupled with the fact that not all of the cell-wall mannan was removed by enzyme action is consistent with the view of Ballou (1974) that yeast cell walls contain a variety of different mannan molecules.

Overall, our results show that a large proportion of the yeast cell-wall mannan is attached to the cell wall through a glucan component since degradation of isolated walls by either (1→3)- β -D-glucanase or (1→6)- β -D-glucanase releases this polysaccharide. On isolation, the alkali-soluble glucan component of the cell wall contains an associated mannan fragment (Fleet & Manners, 1976). This fragment, which probably represents a remnant of the wall mannan component, is released by either (1→3)- β -D- or (1→6)- β -D-glucanase hydrolysis. The other products of glucanase action on isolated cell walls or alkali-soluble glucan preparations are very similar. It appears from these data, therefore, that the alkali-soluble glucan component of the yeast cell wall, originally described by Eddy & Woodhead (1968) and subsequently studied by us (Fleet & Manners, 1976), is closely associated with some parts of the mannan component. Furthermore, the association of mannan and (1→6)- β -D-linked glucan in the products of (1→3)- β -D-glucanase degradation of either cell walls or preparations of alkali-soluble glucan suggests that some (1→6)-linked β -D-glucose residues may be involved in this association of mannan with glucan. If this is so, the detailed chemistry of this association merits investigation, and could perhaps be resolved by a more thorough analysis of the glucan-mannan fragments reported in this study.

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