THE STRUCTURES OF TWO GLUCANS FROM YEAST-CELL WALLS

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On the basis of methylation, periodate oxidation and enzymic degradation studies, Manners and Patterson [1,2] suggested that yeast glucan was a branched polysaccharide consisting of main chains of β-(1→6)-linked D-glucose residues to which linear side chains of β-(1→3)-linked D-glucose residues were attached. A structure of the same general type, but differing in some details, has been proposed independently by Misaki and his co-workers [3,4]. Both of these structures differed significantly from those suggested previously by Bell and Northcote [5] and by Peat and his co-workers [6]. The former suggested that yeast glucan was highly branched, and had β-(1→2)-interchain linkages whilst the latter proposed a linear structure containing certain sequences of β-(1→3)- and β-(1→6)-linked D-glucose residues. Bacon and Farmer [7] have recently suggested that these differences in structure arise, in part, from the heterogeneous nature of yeast glucan, and that it is, in fact, a mixture of two polysaccharides. The minor component of this mixture was identified by infrared spectroscopy as a β-(1→6)-glucan. We now report confirmation of this suggestion, and chemical evidence for the structures of the two glucans.

Pressed baker's yeast (2 kg) was dispersed in 6% sodium hydroxide solution at 18° for 24 hr and after centrifugation, the insoluble material was extracted twice with 3% sodium hydroxide solution at 75° for 3 hr. The combined alkaline extracts contained mannan, which was precipitated by Fehlings solution, and a small amount of glucan which on partial acid hydrolysis, gave glucose and both series of oligosaccharides derived from laminaribiose and gentiobiose. The alkali-insoluble residue was extracted 27 times for 3-hr periods with 0.5 M acetic acid at 90°. The combined acetic acid extracts contained a mixture of glycogen and a glucan which on partial acid hydrolysis gave rise to glucose and gentiosaccharides. The two polysaccharides were separated by selective precipitation of the glycogen as the iodine complex, and final traces of glycogen were removed from the glucan by α-amylolysis. The final yield of glucan was 1.23 g.

The purified polysaccharide is identified as a β-(1→6)-glucan from the following evidence:

(a) it has $[\alpha]_D$ −32° (c 1% in H_2O); compare values of −46°, −38° and −9° for pustulan [8], luteose [9] and laminarin [10] respectively;

(b) it had a degree of polymerisation of 140 ± 10, based on the sorbitol content of an acid hydrolysate of the borohydride-reduced polysaccharide [11];

(c) paper chromatographic analysis showed the presence of glucose, gentiobiose, gentiotriose, gentiotetraose, gentiopentaose and a trace of laminaribiose in a partial acid hydrolysate;

(d) it was not attacked by bacterial or fungal laminarinase preparations, but was readily hydrolysed by a purified endo β-(1→6)-glucanase from *Pencillium brefeldianum* [12] to give glucose and gentiosaccharides and the trisaccharide 3^2-β-glucosylgentiobiose; the enzyme preparation has no action on laminarin;

(e) on oxidation with 0.015 M sodium metaperiodate at 18° in the dark, 2.1 mol. prop. of periodate were reduced per glucose residue;

(f) on application of the Smith degradation procedure (periodate oxidation, borohydride reduction and mild acid hydrolysis), glycerol and glyceryl β-glucoside were amongst the products. The above results show the presence of a high proportion of β-(1→6)-linked glucose residues, together with a small number of β-(1→3)-linked residues.

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The acetic acid-extracted alkali-insoluble residue still contained traces of the β-(1→6)-glucan, which were removed by incubation with the β-(1→6)-glucanase from *P. brefeldianum*. This purification step did not solubilise the residue. This major component of the cell wall was characterised as a branched β-(1→3)-glucan on the basis of the previous results [1,2] and from the following evidence:

(a) a partial acid hydrolysate contained glucose, laminaribiose, laminaritriose, laminaritetraose and a trace of gentiobiose;

(b) a bacterial laminarinase preparation gave a similar mixture of sugars;

(c) on oxidation with sodium metaperiodate, 0.11 mol. prop. of periodate were reduced per glucose residue, and one mol. prop. of formic acid was liberated from approx. 30 glucose residues. Before treatment with the β-(1→6)-glucanase, the glucan reduced 0.16 mol. prop. of periodate, per glucose residue, and liberated one mol. prop. of formic acid from 8 glucose residues;

(d) the glucan had a degree of polymerisation of 1450 ± 100 by the sorbitol method [11].

In the methylation analysis of the original glucan preparation [1,2], 6.4% of tetra-O-methyl D-glucose was obtained, equivalent to the presence of one non-reducing terminal group per 16 glucose residues. Since this original preparation is now recognised as being a mixture of a β-(1→3)-glucan with about 10% of a β-(1→6)-glucan, this tetra-O-methyl D-glucose therefore arises from both polysaccharides. However, the major part is derived from the β-(1→3)-glucan [2]; this indicates that the latter has a branched structure. The new results are in accord with this view but suggest that the degree of branching is much less than originally believed [1–5]. The low degree of branching is consistent with early periodate oxidation studies carried out on insoluble glucan, prepared by a slightly different method, in which a chain length of 28 glucose residues was obtained [13]. It should be emphasised that the observed degree of branching is a statistical value, and that individual chains may vary greatly in length. Nevertheless, the low degree of branching would provide a molecular structure capable of forming a cell-wall "envelope" which could retain the characteristic shape of the cell [14]. Moreover, this relatively open structure could provide a mesh within which other polysaccharides, e.g. the β-(1→6)-glucan and glycogen, could be entrapped.

The present evidence for a mixture of β-(1→6)- and β-(1→3)-glucans is in accord with the results of Peat and his co-workers [6] since partial acid hydrolysis of the above mixture would be expected to give oligosaccharides containing varying numbers of (1→3)- and/or (1→6)-linkages. It should be noted that the present results do not eliminate the possible presence in the β-(1→3)-glucan molecule of glucose residues linked only through C1 and C6, but the relative proportion of these cannot be large in view of the small amount of gentiobiose and the absence of gentiotriose in the partial acid and enzymic hydrolysates of the β-(1→3)-glucan.

Although all the above work was carried out on a strain of *Saccharomyces cerevisiae*, there is evidence that other yeasts contain both the β-(1→3)- and β-(1→6)-glucans as cell-wall components. Using the procedure of Bell and Northcote [5], glucan preparations were isolated from *Kloeckera apiculata, Schizosaccharomyces pombe, Saccharomyces fermentati* and *S. fragilis* by Dr. J.C.Patterson [2]. Acetic acid extraction of these gave a mixture of soluble polysaccharides. After removal of glycogen by α-amylolysis, the residue on partial acid hydrolysis yielded a mixture of glucose and gentiosaccharides.

The presence of alkali-soluble glucan in yeast-cell-wall preparations has been reported by several workers [15,16], and the nature of the material obtained together with the mannan during our original extraction is now being investigated. It is possible that it may be a mixture of low molecular weight material derived from the insoluble branched β-(1→3)-glucan and the more soluble β-(1→6)-glucan.

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