Short Communications

A Re-examination of the Molecular Structure of Yeast Glucan

By D. J. MANNERS and J. C. PATTERSON
Department of Chemistry, University of Edinburgh, and
Department of Brewing and Applied Biochemistry, Heriot-Watt College, Edinburgh

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Although the presence of chains of β-(1→3)-linked D-glucose residues in yeast glucan is well established (Northcote, 1963), the nature and location of other glucosidic linkages is uncertain. On the basis of methylation analysis, Bell & Northcote (1950) suggested that the glucan was highly branched, had β-(1→2)-interchain linkages, and an average chain length of about 9 glucose residues. However, since on partial acid hydrolysis yeast glucan gave rise to a mixture of oligosaccharides containing both β-(1→3)- and β-(1→6)-linkages, Peat, Whelan & Edwards (1958*a*) concluded that the glucan was a linear molecule containing certain sequences of β-(1→3)- and β-(1→6)-linked glucose residues. The presence of (1→6)-linkages in yeast glucan was also confirmed by Peat, Turvey & Evans (1958*b*). We have therefore re-examined the minor structural features of yeast glucan, using a specimen (sample A) prepared by Dr W. D. Annan from the cell walls of baker's yeast according to the method of Bell & Northcote (1950). Our studies were facilitated by Dr D. J. Bell and Professor S. Peat, who kindly supplied samples of their respective preparations (samples B and P).

A branched glucan containing both β-(1→3)- and β-(1→2)-linkages should, on partial hydrolysis, yield laminaribiose and sophorose. The three samples of glucan (about 90mg.) were heated at 100° with 90% (w/w) formic acid for 30min., followed by 0·44N-H_2SO_4 for 1hr. The neutralized hydrolysates were fractionated by preparative paper chromatography, and the individual sugars methylated (by a micro-scale modification of the method of Kuhn, Trischmann & Low, 1955), methanolysed and examined by gas–liquid chromatography (Aspinall, 1963). Each oligosaccharide gave rise to the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucose and of a tri-*O*-methyl-D-glucose, the identity of which enabled the oligosaccharide to be characterized. Authentic samples of laminaribiose, laminaritriose, gentiobiose and sophorose were similarly analysed.

All three samples of glucan gave similar products, namely glucose, laminaribiose, laminaritriose and gentiobiose. Sophorose was not detected, indicating that (1→2)-linkages were absent from the glucans, including sample B. The gentiobiose was not an artifact arising from acid-reversion, since pachyman, an insoluble linear β-(1→3)-glucan kindly provided by Professor W. J. Whelan (Warsi & Whelan, 1957), on partial acid hydrolysis under the above conditions, did not yield gentiobiose.

Glucan A (2g.) was methylated 16 times (with methyl sulphate and NaOH for the first six methylations, and methyl iodide and Ag_2O subsequently) to give a product, having 41·5% of OMe, in a final yield of 23%. Acid hydrolysis of a portion (250mg.) and fractionation on a cellulose column gave the following sugars, which were identified by gas–liquid chromatography of the methyl glycosides, and estimated by hypoiodite oxidation: 2,3,4,6-tetra-*O*-methyl-D-glucose, 6·4%; 2,4,6-tri-*O*-methyl-D-glucose, 63·4%; 2,3,4,-tri-*O*-methyl-D-glucose, 7·5%; 2,4-di-*O*-methyl-D-glucose, 8·3%; 4,6-di-*O*-methyl-D-glucose, 5·8%. In addition, the following sugars were tentatively identified: 3,4,6-tri-*O*-methyl-D-glucose, 0·8%; 2,6-di-*O*-methyl-D-glucose, 5·7%; mono-*O*-methyl-D-glucose and D-glucose, 2·1%. Although methylation was incomplete, the results clearly show that the molecule is branched, with a chain length of about 15 glucose units, and that about 7% of (1→6)-linked residues are present. Owing to undermethylation, the nature of the interchain linkages could not be unambiguously established, but the presence of 8·3% of 2,4-di-*O*-methyl-D-glucose indicates that a significant proportion of glucose residues are triply-linked at C-1, C-3 and C-6. The 3,4,6-tri-, 4,6-di- and lower methylated sugars are believed to arise from undermethylation and not to be structurally significant. The present methylation studies therefore confirm the results of Bell & Northcote (1950) with respect to the branched nature of the molecule, and those of Peat *et al.* (1958*a*) with regard to the presence of (1→6)-linked D-glucose residues.

The proportion of triol groups in glucan A was estimated from the amount of formic acid released on periodate oxidation. The results corresponded to the production of 1mol. of formic acid from

every 7 glucose residues; for glucans B and P, 1 mol. of formic acid was liberated from 10 glucose residues (Bell & Northcote, 1950; Peat *et al.* 1958*a*). The present result of 14% of triol groups is in good agreement with the combined yield of non-reducing end groups (6·4%) and (1→6)-linked glucose residues (7·5%) by methylation analysis.

Additional information on the arrangement of the two types of linkage was obtained by enzymic degradation studies. Incubation of glucan A with a bacterial laminarinase preparation kindly supplied by Glaxo Research Ltd. (Greenford, Middlesex) gave glucose, laminaribiose and laminaritriose, together with about 10% of an enzymically resistant and water-soluble glucan (limit dextrin); gentiobiose was not released. The enzyme preparation did not hydrolyse gentiobiose or the linear β-(1→6)-glucan, pustulan (see Lindberg & McPherson, 1954). The limit dextrin consisted largely of β-(1→6)-linked glucose residues since: (*a*) a partial acid hydrolysate was similar to that from pustulan, and contained a mixture of sugars with the R_{Glc} values of glucose, gentiobiose, gentiotriose and gentiotetraose; (*b*) by methylation analysis, the major tri-*O*-methyl-D-glucose formed was the 2,3,4-isomer; (*c*) the limit dextrin was slowly hydrolysed by a β-(1→6)-glucanase preparation from *Penicillium brefeldianum* QM1872, kindly supplied by Dr E. T. Reese and Dr M. Mandels.

It is therefore concluded that yeast glucan contains main chains of β-(1→6)-linked D-glucose residues to which are attached linear side chains of β-(1→3)-linked D-glucose residues. It seems probable that different samples of glucan differ in the degree of substitution of the main chain, and in the lengths of the side chains. For example, in a sample of glucan examined by Misaki & Smith (1963), almost all the β-(1→6)-linked D-glucose residues carry side chains that contain, on the average, 8 glucose residues. In sample A about half of the glucose residues in the main chains carry side chains, the average length of which is 15 glucose residues, whereas in sample B the side chains contain about 8 glucose residues (cf. Bell & Northcote, 1950).

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Studies on Methylazoxymethanol, the Aglycone of Cycasin: Methylation of Nucleic Acids *in vitro*

By Hiromu Matsumoto and Harry H. Higa
Department of Agricultural Biochemistry, University of Hawaii, Honolulu, Hawaii, U.S.A.

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Cycasin, the azoxyglucoside found in *Cycas circinalis* and *C. revoluta* (Nishida, Kobayashi & Nagahama, 1955; Riggs, 1956), is carcinogenic when fed to rats (Laqueur, Mickelsen, Whiting & Kurland, 1963). The active component is the aglycone, MAM* [$CH_3 \cdot N(O) : N \cdot CH_2 \cdot OH$], and not cycasin itself (Kobayashi & Matsumoto, 1965).

* Abbreviation: MAM, methylazoxymethanol.

There is a striking similarity in the lesions of rats given cycasin and those given dimethylnitrosamine (Laqueur, 1964). Dimethylnitrosamine affects primarily the endoplasmic reticulum of the liver cell (Emmelot & Benedetti, 1960). Liver cells of rats given cycasin show histological evidence of breakdown in the endoplasmic reticular structure, with accompanying decrease in liver RNA content (Williams, 1964). Liver nucleic acids are methyl-