AN ALKALI-SOLUBLE GLUCAN FRACTION FROM THE CELL WALLS OF THE YEAST *SACCHAROMYCES CARLSBERGENSIS*

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1. Introduction

During some recent unsuccessful attempts to extend earlier work on the chemical basis of yeast flocculation [1], we obtained a glucan fraction from the cell walls of certain yeast strains which has not hitherto been described. The preparation and some of the properties of this material are outlined in the present communication.

Cell-wall fractions were prepared from shaken cultures of yeast strain N.C.Y.C.74 * harvested after growth for 18 hours in a liquid malt-extract medium [2]. The cell walls were suspended (about 10 mg dry weight/ml suspension) in 3% (w/v) NaOH solution at 4° under nitrogen for up to 9 days. Portions of the suspension were withdrawn at intervals and the cell-wall residue separated by centrifugation at 1500 X *g* for 15 min. The new glucan fraction was obtained when the supernatant solution was brought to about pH 10 by the addition of glycine. The solution then tended to gel. The material responsible was separated by centrifugation at 1500 X *g* for 30 min and was washed in a relatively large volume of water. It dissolved readily in 3% (w/v) NaOH solution and the glucan fraction precipitated again when the solution was brought to about pH 10. Chromatographic analysis on paper indicated that glucose was the main product of hydrolyzing the precipitate with $2NH_2SO_4$. The progress of the extraction of the glucan fraction, which represented about 20% of the weight of the walls of yeast strain N.C.Y.C.74, is illustrated in fig. 1. Similar materials were detected in the strains designated N.C.Y.C. 1109, 1110 and 1111.

* N.C.Y.C., British National Collection of Yeast Cultures.

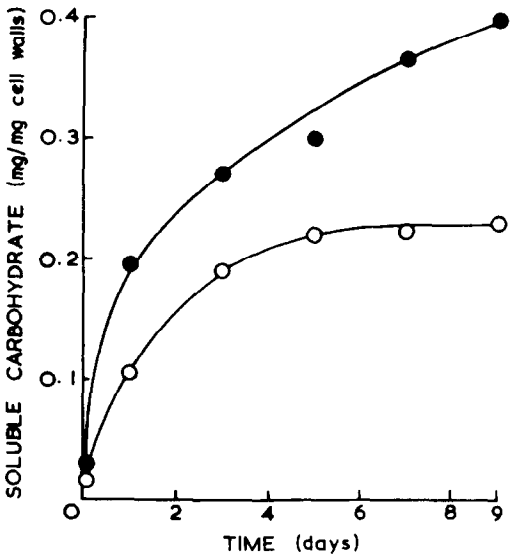

Fig. 1. Mannan and glucan fractions extracted from the yeast cell walls by 3% (w/v) aqueous NaOH at 4° under nitrogen during several days. •, The alkaline extract was boiled for 10 min and the mannan was precipitated as a copper complex [2]; ○, glucan precipitated when the (unboiled) alkaline extract was brought to pH 9 with acetic acid. Both carbohydrates were assayed with the anthrone reagent [4].

Fig. 1 also shows how the mannan content [2] of the supernatant solution varied with the duration of the extraction.

The products of extracting the cell walls included various minor components which separated from the fractions illustrated in fig. 1 when the neutralized extracts were dialyzed against distilled water. The diffusate contained both polypeptides [3] and carbohydrates [4], the latter being equivalent to about 4%

of the dry weight of the cell walls. The same amount of material in these two categories was present after extraction for 1 or 9 days.

2. Properties of the glucan fraction

The glucan fraction contained only a relatively small amount of polypeptide [3]. $[\alpha]_D^{24}$ was about $+9^o$ for a solution in 3% (w/v) aqueous NaOH. The material was not hydrolysed by a mixture of α- and β-amylases. It therefore probably contained β-linked glucose residues, as does the more familiar insoluble yeast glucan.

Alkaline solutions of the glucan fraction exhibited a single boundary during ultracentrifugation. The molecular weight, corrected to zero glucan concentration, was estimated to be $5.2 \times 10^5 \pm 1.2 \times 10^5$ (95% confidence limits). An analogous material extracted from the so-called aberrant cell walls formed by the spheroplasts [5] of the same yeast strain exhibited a molecular weight of $1.1 \times 10^5 \pm 0.3 \times 10^5$ (95% confidence limits).

When the glucan fraction was kept in alkaline solution at 100^o for 15 min it subsequently failed either (1) to sediment as a discrete boundary during ultracentrifugation or (2) to gelate at pH 7. Similar changes occurred during several days at 20^o. It may be relevant that alkaline solutions of certain β-glucans have previously been shown to undergo spontaneous degradative changes [6].

Samples of the glucan fraction were negatively stained with uranyl acetate and examined in an electron microscope. They appeared amorphous under conditions where the spheroplast cell walls themselves were clearly fibrous as previously indicated [5]. On the other hand 2 out of 5 preparations of the precipitated glucan fraction from the spheroplasts appeared to contain fibres.

3. Relation to earlier work

Warm solutions of NaOH are known to dissolve some of the glucose residues found in the yeast cell wall, though the chemical nature of this soluble fraction has not been established [7]. The present observations suggest that some of these glucose residues may represent a degraded form of the glucan fraction dissolved at 4^o. The latter may conceivably be complexed with mannan in the cell wall. Another interesting possibility is that the glucan fraction obtained at 4^o represents a metabolic precursor of the insoluble yeast glucan, which is presumably a much larger molecule.

Acknowledgement

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