High molecular weight precursors of glucans in *Saccharomyces cerevisiae*

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Abstract

Nascent β-1,3 glucan synthesized by mixed membrane fractions from *Saccharomyces cerevisiae* was solubilized by extraction with hot SDS or urea. Nature of the material was analyzed by electrophoresis and gel filtration. As determined by gel filtration, Mr of synthesized glucans exceeded 1,500 kDa, but was below 20,000 kDa. This nascent material served as an acceptor for further glucose transfer reactions, giving rise to glucan molecules over 20,000 kDa. It is suggested that the high Mr precursor components represent protein-bound glucan molecules in transit to the cell surface.

Introduction

Beta glucans constitute the most important structural polysaccharides of the yeast cell wall. Starting from the first demonstration of β-1,3, β-1,6 glucan synthesis *in vitro* by *Saccharomyces cerevisiae* (Lopez-Romero & Ruiz-Herrera 1977), we have witnessed great advances in our understanding of the mechanism of glucan biosynthesis; e.g. fibrillogenesis *in vitro* (Larriba et al. 1981), the role of nucleotides (Notario et al. 1982, Szaniszlo et al. 1985), and the dissociation of the glucan synthetase into catalytic and regulatory subunits (Kang & Cabib 1986) (see review by Ruiz-Herrera 1991).

Previous results from our groups have demonstrated that synthesis of β-1,3 glucan in *S. cerevisiae* and *Candida albicans* occurs over an acceptor of protein nature (Andaluz et al. 1986, 1988). Membrane samples from either fungus synthesized glucan components which could be extracted with hot SDS, and separated into a fraction Mr ca. 80 kDa, probably representing the protein acceptor bound to a few glucose molecules, and a higher Mr fraction, probably corresponding to larger chains of glucan molecules, still attached to the precursor protein. Both fractions were sensitive to hydrolysis by proteases and exo β-1,3 glucanases.

In the study here reported we have proceeded to solubilize nascent glucans with either SDS or urea, and to study their characteristics by means of electrophoresis and gel filtration.

Materials and methods

Strain and culture conditions

*Saccharomyces cerevisiae* X2180-1A was maintained on slants of solid YED medium (1% yeast extract, 1% glucose, 2% agar). Cells from these slants were inoculated into 25 ml liquid YED, and incubated for 10–12 h at 28 °C with shaking (150 rpm). This culture was used as inoculum (initial absorbancy, 0.001 A at 600 nm, by calculation) for 500 ml of liquid YED. The cultures were incubated as above to reach a final A at 600 nm of 1.0–1.5 (usually 12–13 h).

Isolation of mixed membrane fractions

Cells were harvested by centrifugation, and washed by centrifugation once with distilled water, once with
50 mM Tris HCl buffer pH 7.8, and once with 1 M sucrose in Tris buffer. They were resuspended in 15 ml of 1 M sucrose in Tris buffer, mixed with an equal volume of glass beads (0.45–0.5 mm in diameter), and broken with a Braun cell homogenizer. The extract was centrifuged at 10,000 × g to remove cell walls. The supernatant was recovered and centrifuged at 50,000 × g for 1 h. The sediment, designated as mixed membrane fraction (MMF), was homogenized in 4 ml of Tris-1 M sucrose, distributed into aliquots, and kept at -80º C, until further used.

Synthesis of glucan in vitro

Incubation mixtures contained in a final volume of 5 ml, 50 mM Tris HCl pH 7.8, 1 M sucrose, 600 mg bacterial α-amylase type II (Sigma), 7.5 μCi uridine diphosphate glucose (UDP-[U-14C-Glc]) (Amersham, carrier-free, 252 mCi/mmol), and 2 ml of MMF prepared as above described. Samples were incubated at 24º C during 4–8 min. Ten ml of 10% trichloroacetic acid (TCA) were added to stop the reaction, and the samples were left for at least 1 h in an ice bath, centrifuged at 11,000 × g, washed twice by centrifugation with 10% TCA, twice with 50% ethanol, and twice with water.

Solubilization of nascent glucans

Samples of incubation mixtures treated as described above, were mixed with 1 ml of solubilizing agent, and placed in Eppendorf tubes. Samples were heated in a boiling water bath for 5 min, and centrifuged at 11,000 × g for 15 min. Supernatant was recovered and kept at -80º C until further used. Aliquots were placed over glass fiber disks, and dried. Radioactivity was counted in a liquid scintillation spectrometer.

Electrophoresis

Glucan samples were treated with 8 M urea and centrifuged as described in the preceding paragraph. Supernatants were made with two volumes of solubilizing mixture (8% SDS, 40% glycerol and 20% mercaptoethanol) and heated for 10 min in a boiling water bath. After centrifugation as described above, the supernatant was subjected to electrophoresis using normal tubes for vertical disc electrophoresis. Samples (0.3 ml), were placed on top of tubes containing successive layers of the following solutions and gels (in Tris HCl buffer pH 8.0 containing 2% SDS, starting from the anode): 0.4 ml 8% polyacrylamide, 0.4 ml 3% polyacrylamide, 0.4 ml 0.5% agarose, 0.5 ml 0.25% agarose, 0.5 ml 25% glycerol. Sample was covered with 0.5 ml Tris HCl pH 6.8. Electrophoresis towards the anode was carried out in a cell for vertical electrophoresis for 2 h at 2 mA per tube, and 4 h more at 4 mA per tube. After electrophoresis, liquid samples were carefully removed with a syringe, mixed with 1.5 ml water and 10 ml scintillation fluid. Their radioactivity was measured with a scintillation spectrometer. Gels were cut in fractions, digested with 30% H2O2-NH4OH (10:1 by vol.) at 80º C, mixed with 1.5 ml water and 10 ml scintillation fluid, and their radioactivity was measured as above. A control which remained without electric current for the same time as the electrophoresed sample was simultaneously run.

Paper chromatography

Glucan samples extracted with different solvents as described above, were streaked 10 cm from the origin of 56 cm-long Whatman 3 MM strips, and irrigated overnight with a mixture of 1 M acetic acid:ethanol (30:70, by vol.). After air-drying, 2 cm-long segments were cut, and their radioactivity was counted.

Column chromatography

Columns of BioGel A 1.5 M (BioRad, 1.4 cm in diameter, and 39 cm in height), and Sepharose 2B (Pharmacia, 1.6 × 33 cm) were used. They were equilibrated and eluted (4 ml per h) with 8 M urea containing 0.05% mercaptoethanol, and 1-ml fractions were recovered. Aliquots were dried over glass fiber disks, and their radioactivity was measured as above. Void volume (Vo) was measured with blue dextran (Pharmacia), and measuring A at 280 nm.

Miscellaneous

α-Glucanase free from β-glucanase; and exo β-1,3 glucanase free from β-glucosidase, β-1,6 glucanase and α-1,3 glucanase were purified as described previously (Andaluz et al. 1986). Reported results are representative of experiments performed at least twice.