Two Pools of Glycogen in *Saccharomyces*

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The effect of different extraction procedures on the yields of water-soluble and water-insoluble glycogen fractions from a number of *Saccharomyces* strains was studied by using a specific method for glycogen determination. The similarity of the yields obtained by the different procedures showed that neither form of glycogen is an artifact, and variations in the relative amounts of glycogen in the two fractions during cell growth and in different yeast strains suggest that they represent different pools of storage material with specific roles in cell development and differentiation. A proportion of the water-insoluble glycogen fraction, solubilized by mechanical agitation, was shown to be strongly associated with a β-glucan-like polysaccharide that may be a cell wall component.

Although glycogen has long been known as the storage polysaccharide in yeast cells, and many procedures have been described for its extraction and estimation (11, 13, 18, 27), the cellular location of the polysaccharide remains unclear. Two glycogen fractions are obtained after treating yeast cells with hot alkali: a minor water-soluble fraction and a major water-insoluble fraction, which is solubilized by repeated extractions with hot acid (18, 22). The insolubility of the major glycogen fraction indicated that it may be associated with the cell wall (11, 13, 27), but Northcote (18) reported that all yeast glycogen was released, provided the cell was mechanically disrupted, and he concluded that yeast cells contain only one type of storage glycogen that does not act as a structural component of the cell wall. He ascribed the apparent insolubility of the major glycogen fraction in alkali-treated cells to the physical entrapment of high-molecular-weight glycogen within an insoluble cell-wall glucan membrane (18). More recently, it was reported that brief sonication of a suspension of yeast cells in alkali before heating was sufficient to rupture this membrane, so that all glycogen was recovered in solution (25).

Evidence has been reported from this laboratory that the water-insoluble glycogen of yeast cells may be located outside the cytoplasmic membrane in association with cell wall components (9). Here we report results, obtained in a comparison of procedures for the extraction and quantitation of glycogen from various yeast strains, which lend support to the existence of two different pools of glycogen in *Saccharomyces*, and which indicate that the insoluble glycogen component is associated via a stable linkage to a component of the cell wall.

**MATERIALS AND METHODS**

Salivary α-amylase, rabbit liver glycogen, and shellfish glycogen were purchased from the Sigma Chemical Co., St. Louis, Mo. The glycogen was purified by three precipitations from 70% ethanol solution. *Aspergillus niger* amylglucosidase (glucoamylase) was prepared by the method of Fleming and Stone (Biochem. J. 97:13p, 1965), the second enzyme peak from a diethylaminoethyl-cellulose fractionation being used. Concanavalin A-Sepharose 4B conjugate and *Cytophaga* endo-1,3-β-D-glucanohydrolase were prepared and kindly made available by S. D. Killies and J. J. Marshall, respectively, both of the Department of Biochemistry, University of Miami. Peptone, yeast extract, and malt extract were purchased from Difco Laboratories, Detroit, Mich.

Total polysaccharide was determined by the phenol-sulfuric acid method (4) or by hydrolyzing the polysaccharide in 1.5 N sulfuric acid for 3 h at 100°C and then determining the amount of reducing sugars or glucose released. Reducing sugars were determined by a modified copper reducing method (23), and glucose was determined by a specific glucose oxidase method (14).

The optical rotation of polysaccharide and sucrose solutions was measured at 23°C in a 2-cm cell with a spectropolarimeter (Carey 60). These measurements were kindly made by A. H. Brady, Department of Medicine, University of Miami.

**Determination of glycogen.** In routine glycogen determinations, yeast cells were weighed (wet weight) in clinical centrifuge tubes (12-ml volume) and suspended in 20% potassium hydroxide solution (0.05 to 0.40 g/ml). The tubes were immersed in a boiling-water bath for 1 h and, after cooling, the suspensions were adjusted to pH 6 to 7 with 5 N hydrochloric acid. Two volumes of ethanol were added, and the resulting precipitates were recovered by centrifugation, washed three times with 67% ethanol, and freed of excess ethanol by inverting the centrifuge tubes over filter paper for about 5 min. The precipitates were suspended in water (2 ml).
with gentle warming, with use of a Vortex mixer to ensure that a fine suspension was obtained. Portions of the suspensions, and of the supernatant solutions after centrifugation (14,000 x g), containing 50 to 100 μg of glycogen were incubated at 37°C for 2 h in digests (1 ml) containing A. niger glucoamylase (2 IU) and salivary α-amylase (1 IU), 5 mM calcium chloride, and 50 mM sodium acetate buffer, pH 5.0. Glycogen was estimated from the amount of glucose released. The amounts of glycogen found in the suspension and the supernatant solution represent the total glycogen and water-soluble glycogen, respectively. Water-insoluble glycogen was determined from the difference between these values.

Organisms and media. All the parent strains of Saccharomyces used in this study were kindly made available by F. J. Roth, Jr., of the Department of Microbiology, University of Miami. Mutant strains were induced and selected as described by Chester (3).

Cells were grown routinely in medium containing glucose (1%), peptone (0.5%), yeast extract (0.3%), and malt extract (0.3%). After autoclaving, the pH was about 6.7. The medium was inoculated with a starting culture (10% by volume) and incubated at 22°C on a rotary shaker. The cells were collected by centrifugation at 13,000 x g and washed three times with water at 4°C before glycogen determinations were made. The dry weight of cells was determined by filtering a portion of the culture media through a preweighed membrane filter (0.45-μm pore size, Millipore Corp.) and drying the filter to constant weight over P_2O_5 in a vacuum oven at 50°C.

To obtain high glycogen contents, resting cells were suspended for 2 h in 0.1 M glucose medium under the conditions described by Trevelyan and Harrison (28). Yeast cells were fixed by suspending them at 22°C in 1.5% glutaraldehyde solution at pH 6.5 for 2 min. Excess glutaraldehyde was removed by washing the cells three times with water.

Alkali treatment of yeast cells. Washed cells (10.5 g [wet weight]) were suspended in 20% potassium hydroxide solution (23 ml) and heated at 100°C for 1 h. The suspension was cooled and brought to pH 7.0 with 5 N hydrochloric acid, and 2 volumes of 95% ethanol was added in small portions with efficient mixing. After standing at 4°C for 4 h, insoluble material was recovered by centrifugation and washed three times with 67% ethanol. The washed residue was suspended in water (16 ml), warmed at 70°C with shaking for 5 min, and recovered by centrifugation. The residue was similarly extracted with water two more times.

Homogenization of yeast cells. Fresh or glutaraldehyde-treated cells (5 g [wet weight]) were washed and suspended in buffer as described by Mill (16), except that octanol was omitted from the buffer. The cell suspension was shaken twice for 2 min with glass beads (4.5 g, 0.45 to 0.55 mm in diameter) in a Braun cell homogenizer (Bronwill Scientific Inc., Rochester, N.Y.) that was cooled with liquid carbon dioxide to prevent the temperature from rising above 15°C. The homogenate was decanted from the glass beads, which were washed three times with water (8 ml). Portions of the combined washings and homogenate were used to determine total glycogen and were then centrifuged for 30 min at 37,000 x g. Soluble glycogen was determined in the clear supernatant solution obtained.

Fresh cells (5.5 g [wet weight]) were ground for 10 min under liquid nitrogen with a stainless-steel pestle and mortar. The homogenate was suspended in water, heated at 100°C for 10 min, and then centrifuged at 5,000 x g and at 35,000 x g.

Homogenization of water-insoluble residue from alkali-treated cells. Insoluble yeast residue (4.5 g [wet weight]) containing 51 mg of glycogen (Table 4, fraction A) was suspended in buffer solution (8 ml), pH 7.2, containing 150 mM sodium phosphate and 10% mannitol. The suspension was shaken with glass beads (4.5 g) in a Braun homogenizer as described above. The suspension was shaken twice for 2-min periods and then centrifuged at 23,000 x g for 20 min. The insoluble sediment was twice washed with water (10 ml), and 2 volumes of 95% ethanol was added to the combined supernatant and wash solutions (34 ml) to precipitate the water-soluble polysaccharide. The precipitated polysaccharide was washed three times with 67% ethanol and redisolved in water (3 ml) for determinations of total polysaccharide and glycogen (Table 4, fractions B and C).

Fractionation of solubilized polysaccharide on concanavalin A-Sepharose column. Concanavalin A-Sepharose conjugate was placed in the barrel of a small syringe (2.5 ml) and washed with a 2 mM potassium phosphate-0.1% sodium chloride solution (pH 7.0) containing rabbit liver glycogen (0.2 mg/ml) to remove unbound concanavalin A. The column was then washed with buffer containing methyl-α-D-glucoside (10 mg/ml) to remove bound glycogen and finally washed exhaustively with buffer until no phenol-sulfuric acid-positive material was detected in the eluate. Water-soluble polysaccharide released by homogenization of the insoluble yeast residue was applied to the concanavalin A column, and the column was irrigated with buffer solution at a flow rate of 5 ml/h. The total polysaccharide in the eluted fractions was determined by the phenol-sulfuric acid method. The column was then irrigated with buffer containing methyl-α-D-glucoside (10 mg/ml), portions (0.05 ml) of the eluted fractions were mixed with 0.02% iodine-0.2% potassium iodide solution, pH about 4.0 (0.4 ml), and the absorbance was measured at 490 nm to test for the presence of glycogen (Fig. 1). Fractions containing glycogen were combined, exhaustively dialyzed against water to remove methyl-α-D-glucoside, and the total polysaccharide and glycogen were determined (Table 4, fraction E).

Glucoamylase degradation of eluted glycogen fraction. The dialyzed glycogen fraction eluted from the concanavalin A-Sepharose column was incubated in a cellulose dialysis bag with A. niger glucoamylase (2 IU/ml), salivary α-amylase (1 IU/ml), 5 mM calcium chloride, and 50 mM sodium acetate buffer solution, pH 5.0 (20 ml). The bag was immersed in a stirred solution (50 ml) of 50 mM sodium acetate, pH 5.0, maintained at 37°C, and samples of the external buffer were removed at in-
tervals for determinations of the glucose released into the dialysate (Table 2, fraction F). After 3 h of incubation, the contents of the dialysis bag were exhaustively dialyzed twice against water (40 ml), heated at 100°C for 5 min to inactivate the enzyme, and freeze-dried. The residue was dissolved in a small volume of water (7 ml), the total polysaccharide concentration was determined (impermeate, Table 4, fraction F), and the solution was applied to a concanavalin A-Sepharose column as previously described. The total polysaccharide in the fractions eluted with 2 mM potassium phosphate-0.1% sodium chloride buffer, pH 7.0, was determined by the phenol-sulfuric acid method (Table 4, fraction G).

RESULTS

Extraction of water-soluble glycogen component. Constant amounts of soluble and insoluble glycogen were recovered from yeast cells after heating for different times in 20% potassium hydroxide solution (Table 1, experiments 1 and 2) and also after heating for the same time in different concentrations of alkali (Table 1, experiment 3). Essentially the same proportions of soluble and insoluble glycogen were recovered when from 1 to 4 g (wet weight) of yeast cells were extracted in the same volume (15 ml) of 20% potassium hydroxide. Exogenous soluble shellfish glycogen (1 mg), added to yeast cells (1 g) before extraction was recovered in 95% yield after hot alkali treatment (not shown). Neither water-soluble nor water-insoluble glycogen was detected by the specific glucoamylase-glucose oxidase assay procedure when cell suspensions were heated in water (100°C) for 60 min (Table 1, experiment 2) or sonicated at 22°C for 2 min, but about 25% of the total cell glycogen was released into solution when sonication was maintained for 20 min (not shown). Further sonication (45 min) failed to release additional glycogen.

When glutaraldehyde-fixed cells were fractured in a Braun homogenizer, the yields of soluble and insoluble glycogen were similar to those in the hot alkali extraction procedure (Table 2). The same amount of soluble glycogen was recovered when cells were fractured without prior treatment with glutaraldehyde, but the total glycogen content was not determined in these cells. Untreated yeast cells were also fractured by grinding them under liquid nitrogen for 10 min and then suspended and heated in water at 100°C for 10 min to inactivate glycogen-metabolizing enzymes. Centrifugation at 5,000 × g for 10 min did not sediment a fine suspension, and analysis of this suspension revealed that it contained 37% of the total glycogen. However, when the solution was clarified by centrifugation for 10 min at 25,000 × g the amount of soluble glycogen, determined, on a portion of the clear solution removed from above the small sedimented pellet, was similar to that found for cells that had been subjected to the hot alkali procedure (Table 2). Sonication of the cells in 6% KOH solution for two 10-s periods before heating, as described by Rothman and Cabib (25), did not increase the total recovery of glycogen or change the relative proportions of the soluble and insoluble fractions.

Microscopic examination confirmed that the yeast cells were fragmented by the hot alkali treatment. Glucoamylase treatment had no effect on the iodine-staining properties of glycogen in unbroken cells but completely destroyed the iodine-staining capacity of the alkali-treated cells, indicating that all cellular glycogen is susceptible to enzyme action after disruption of the cells.

Glycogen synthesis during cell growth. The 20% alkali extraction procedure followed by the specific enzymatic assay was used to determine the synthesis of water-soluble and water-insoluble glycogen during growth of Saccharomyces cerevisiae (Fig. 2). Rapid synthesis of glycogen ensued at about pH 5.0 (culture medium), the total glycogen content approaching a maximum (1.5% of cell dry weight), and the medium pH reaching a minimum (4.70 to 4.75), after 12.5 h of growth. The subsequent rise in pH was always accompanied by a decrease in cellular glycogen, with only a slow increase in cell mass, but after the culture reached about pH 5.25, more insoluble glycogen was synthesized and the apparent rate of cell growth was increased. These changes in culture pH and cellular growth rate are unlikely to be directly related and probably reflect the adaption of the

<table>
<thead>
<tr>
<th>Table 1. Recovery of glycogen from heated (100°C) yeast cell suspensions in water and in alkaline solutions</th>
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<tr>
<td>Cells</td>
</tr>
<tr>
<td>Expt 1: S. cerevisiae</td>
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<td></td>
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<td>Expt 2: S. cerevisiae</td>
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<td></td>
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<td>Water</td>
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<td>Expt 3: S. ellipsoid</td>
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TABLE 2. Comparison of the amounts of water-soluble glycogen extracted by hot alkali treatment and by mechanical disruption of S. cerevisiae cells

<table>
<thead>
<tr>
<th>Cell Treatment</th>
<th>Glycogen (mg/g [wet wt])</th>
<th>Soluble glycogen (% total)</th>
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<tbody>
<tr>
<td></td>
<td>Soluble</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Expt 1: Glutaraldehyde-fixed cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heated in 20% KOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.56</td>
<td>4.20</td>
</tr>
<tr>
<td>Disrupted in homogenizer&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.50</td>
<td>4.02</td>
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<tr>
<td>Expt 2:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heated in 20% KOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.42</td>
<td>4.22</td>
</tr>
<tr>
<td>Ground in liquid nitrogen&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centrifuged at 5,000 × g</td>
<td>2.09</td>
<td>3.54</td>
</tr>
<tr>
<td>Centrifuged at 35,000 × g</td>
<td>1.31</td>
<td>4.39</td>
</tr>
<tr>
<td>Expt 3:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heated in 6% KOH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.12</td>
<td>4.63</td>
</tr>
<tr>
<td>Sonicated and heated in 6% KOH&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.16</td>
<td>4.34</td>
</tr>
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</table>

<sup>a</sup> Heated at 100°C for 60 min.
<sup>b</sup> See text for details.
<sup>c</sup> The alkaline cell suspension was twice sonicated for 10 s before heating at 100°C for 60 min.

![Fig. 1. Elution from a concanavalin A-Sepharose affinity column of (a) solubilized yeast glucan complex and (b) the glucoamylase-resistant component of the solubilized yeast glucan complex. Symbols: Total polysaccharide (phenol-sulfuric acid method) (■), glycogen iodine stain (absorbance at 490 nm) (●). The arrow in (a) indicates the point at which buffer containing methyl-a-D-glucoside (10 mg/ml) was applied. For other details, see text.](image)

The relative proportions of soluble and insoluble glycogen varied throughout the growth period. Thus, soluble glycogen represented 9% of the total after 12.5 h, increased to 25% after 15 h, and decreased significantly at later stages of growth (25 h). Thereafter, the proportion of soluble glycogen remained low despite an increasing synthesis of insoluble glycogen (Fig. 2). The apparent lag in the synthesis of the soluble glycogen component (12.5 h) was usually not evident or was less marked than shown in Fig. 2. Significant decreases in the proportion of soluble glycogen were also observed during growth of *S. cerevisiae* in 3% glucose medium and during growth of *S. carlsbergensis* and a low glycogen mutant of *S. cerevisiae* under standard conditions (Table 3, cf. 15- and 38- or 40-h culture periods). *S. carlsbergensis* and *S. ellipsoid* contained more glycogen than *S. cerevisiae* when grown for about 20 h under standard conditions (Table 3), and in both cases a greater proportion of the total glycogen was soluble. Incubation of resting *S. cerevisiae* cells in glucose medium for 2 h increased their total glycogen content almost ninefold and increased twofold the proportion of soluble glycogen (40%). The decreased synthesis of total glycogen in the low glycogen mutant strain, however, resulted in an increased proportion of the soluble fraction (Table 3).

Characterization of the polysaccharide released by homogenization of alkali-treated yeast residue. About 30% of the glycogen (polysaccharide degraded by glucoamylase) associated with the hot alkali-treated yeast residue appeared in the supernatant solution after agitation of the residue with glass beads in a Braun homogenizer (Table 4, fraction B). Based on the carbohydrate content, as determined with phenol-sulfuric acid, glucoamylase released 60% of the carbohydrate as glucose. Acid hydrolysis released 90% of the carbohydrate, whether measured specifically as glucose or by the nonspecific reducing sugar assay (Table 4, fraction C). *Cytophaga* endo-(1 → 3)-d-glucanohydrolase degraded the same polysaccharide fraction with the release of reducing sugars but, under similar conditions, this enzyme preparation exhibited little action on rabbit liver glycogen (Table 5).
Almost all of the solubilized yeast polysaccharide was absorbed when a portion of the supernatant solution of the homogenate was applied to a concanavalin A-Sepharose column, and 93% of the applied polysaccharide was eluted, in a fraction possessing an iodine stain characteristic of glycogen, when the column was irrigated with a solution of methyl-α-D-glucoside (Fig. 1a). Analysis of the eluted polysaccharide revealed that the glucan component resistant to the action of glucoamylase was coeluted in the same proportion (40%) as it was present in the polysaccharide solution applied to the column (Table 4, fraction E). A sample of rabbit liver glycogen, which had been similarly absorbed to and eluted from the concanavalin A column, was degraded more than 98% by glucoamylase action and exhibited a positive optical rotation higher than that of the soluble yeast glucan (Table 5). The glucoamylase-resistant component of the yeast glucan was impermeable to cellophane dialysis tubing and
about 85% was not absorbed when reapplied to the concanavalin A affinity column (Table 4, fractions F and G).

A synthetic mixture of the soluble nonglycogen component of the yeast glucan (1 mg) and rabbit liver glycogen (2 mg, previously absorbed to and eluted from a similar affinity column) was readily fractionated on the concanavalin A column. About 70% of the yeast polysaccharide appeared in the unretarded fraction, whereas all of the glycogen component was absorbed by the column (not shown). In a preliminary study, partial acidic hydrolysis of the un retarded glucan fraction and separation of the products by paper chromatography gave a chromatographic pattern different from that of rabbit liver glycogen similarly hydrolyzed. A number of the hydrolysis products corresponded in \( R_f \) to partial acidic hydrolysis products from laminarin, but there was insufficient material for characterization of these products.

**DISCUSSION**

The constant amounts of water-soluble and water-insoluble glycogen recovered from yeast under various alkali extraction conditions (Tables 1 and 2) show that neither glycogen fraction is artifactual. Our failure to increase the solubilization of glycogen by sonication before hot alkali treatment (Table 2) contrasts with the report that all yeast glycogen is released by this procedure (25). Also, the presence of major water-insoluble glycogen fractions in the homogenates of mechanically disrupted yeast cells (Table 2) is at variance with the report that all glycogen is solubilized when cells are mechanically ruptured by, for example, grinding them with sand (8). On the other hand, our results support earlier findings that about 70% of yeast glycogen was not solubilized when cells were ground with sand (11), and that insoluble glycogen, associated with a small particle fraction, sedimented between 1,500 and 14,000 \( \times g \) after disruption of yeast cells in a Mickle cell disintegrator (20). The descriptions of the insoluble glycogen fractions in both of these reports resemble our observation of finely suspended material in the supernatant solution (centrifuged at 5,000 \( \times g \) ) of the homogenate obtained by grinding yeast cells under liquid nitrogen (Table 2). The similarity of the results we obtained by means of alkali treatment or mechanical disruption (Table 2) confirms the early reports that yeast cells contain two pools of glycogen, one readily water soluble and a second, resistant to extraction with hot alkali, that is solubilized only with difficulty.

The contrasting reports on the solubilization of yeast glycogen may be related to the different states of the cells used for extraction. The lower yield of soluble glycogen obtained by hot alkali treatment of pressed yeast, compared with that obtained from the same yeast after drying (8), would be expected if partial hydrolysis of the insoluble glycogen component occurs during the drying process (autolysis). Overestimation of soluble glycogen would also result if such hydrolysis occurs during cell homogenization and if the solutions used for determination of the soluble glycogen component contain insoluble glycogen associated with finely divided, suspended material. To avoid such errors in this study, only fresh yeast cells were used, and glycogen hydrolysis was minimized by fixing yeast cells with glutaraldehyde before homogenization (Braun homogenizer), or by immediately heating the homogenate (liquid nitrogen) at 100°C to inactivate hydrolytic enzymes. In
addition, all soluble glycogen determinations were conducted on solutions that were clarified, if necessary, by centrifuging; this precaution was not taken in at least one study reporting the complete solubilization of yeast glycogen (25).

Northcote (18) suggested that water-insoluble glycogen, remaining after hot alkali treatment of yeast cells, represents a fraction of soluble cytoplasmic glycogen molecules that are too large to diffuse through the interstices of the β-glucan cell wall of the yeast cell. It was assumed that the cell wall envelope was intact because it retained the original cell shape after alkali treatment. However, retention of cell shape is not necessarily indicative of the integrity of the cell wall. Studies of Bauer et al. (2) demonstrated that treatment of yeast cells with an exo-β-(1→3)-glucanase introduces lesions in the cell wall that are not visible in electron micrographs, but through which the intact cytoplasmic membrane and its contents extrude to form a protoplast. The shape of the remaining cell wall envelope is indistinguishable from that of the original cell.

It is unlikely, therefore, that the alkali-insoluble glycogen of the yeast cell is physically entrapped within an intact cell wall envelope. More probably, it is associated with a component of the envelope by a linkage resistant to alkaline degradation and susceptible to subsequent acidic or mechanical degradation. The rapid and preferential degradation of the insoluble glycogen component during formation of yeast cell spheroplasts indicates that this glycogen fraction is located in the periplasmic space outside the cytoplasmic membrane (9), but gives no clue as to the nature of its association with cell wall components. The parallel absorption and elution on a concanavalin A affinity column of the glycogen and nonglycogen components of the glucan complex solubilized by homogenization of the alkali-treated yeast residue (Fig. 1, Table 4), however, gives evidence of the existence of a stable, alkali-resistant bond between the two polysaccharide components. The low $\left[\alpha,\beta\right]$ of this glucan complex and its significant degradation by β-glucanase (Table 5) indicate that many of the linkages of the nonglycogen component are in the β configuration. The failure of a large proportion of the glucoamylase-resistant component of the complex (fraction G, Table 4) to absorb to the concanavalin A column, even when mixed with an equal weight of rabbit liver glycogen (not shown), establishes that the residual glucan does not possess an inherent affinity for concanavalin A and is consistent with the documented failure of β-linked glucosyl residues to interact with the lectin (7, 8). Additional evidence for the presence of an alkali-resistant, and possibly covalent, linkage between the glycogen and a β-glucan component of the yeast cell wall will be reported elsewhere.

The presence of two glycogen fractions in all the yeast strains tested (Table 3) and the invariably increased proportion of the insoluble fraction in aged cells (Fig. 2, Table 3) indicate that the metabolic regulation of the two glycogen pools may be independent. An increase in insoluble carbohydrate has been reported in nondividing yeast cells that are increasing in size during the period preceding the formation of visible ascosporas (27), and acetic acid-soluble (alkali-insoluble) glycogen has been shown to accumulate during sporulation (1). The observed increase in insoluble glycogen during aging of yeast cell cultures (Fig. 2), thus, may be related to the differentiation process, and the two glycogen fractions, although not wholly independent, may represent pools of storage material with specific roles in the metabolism of the yeast cell.

It remains to be established whether the soluble and insoluble glycogen pools studied here correspond to the metabolically distinct "fermentable" and "oxidizable" glycogen pools previously reported to be present in yeast (5). However, our results and earlier reports that the glycogen granules in yeast cells are located in membranous vesicles (19), that large glycogen granules are present in the cell periphery of a mutant yeast strain (6), and that the content of acid-soluble (alkali-insoluble) glycogen is related to flocculation of yeast cells (21) all indicate that there is a real compartmentation of yeast glycogen reserves and that glycogen may play an important role in cell wall metabolism.

Reports of two metabolic pools of glycogen in Dictyostelium discoideum (10), the detection of glycogen in membrane-bound vacuoles (17) and

![Fig. 2. Growth curve of Saccharomyces cerevisiae. Cells were grown under the conditions described in Materials and Methods. Symbols: Soluble glycogen (□), insoluble glycogen (■), glucose (○), pH (●), cell dry weight (△).](http://jb.asm.org/Downloaded from)
in the cell wall of this organism (29), and reports of amyllose in the cell walls of Cryptococcus (12) and other fungi (15) suggest that α-glucans also may have a dual function in a wide range of eucaryotic microorganisms.

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LITERATURE CITED