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Variation of (1→3)-β-Glucanases in *Saccharomyces cerevisiae* During Vegetative Growth, Conjugation, and Sporulation

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The total (1→3)-β-glucanase activities associated with cell extracts and cell walls of *Saccharomyces cerevisiae* were measured during vegetative growth, conjugation, and sporulation. Using a system of column chromatography, we resolved (1→3)-β-glucanase activity into six different enzymes (namely, glucanases I, II, IIIA, IIIB, IV, and V). The contributions of the individual enzymes to the total activity at the different stages of the life cycle were determined. Total glucanase activity increased during exponential growth and decreased in stationary resting-phase cells. Glucanase IIIA was the predominant enzyme in stationary resting-phase cells. Glucanases I, II, IIIB, and IV were either absent or present at low levels in stationary phase cells, but their individual activities (in particular, glucanase IIIB activity) increased substantially during exponential growth. Total (1→3)-β-glucanase activity did not change significantly during conjugation of two haploid mating strains, *S. cerevisiae* 2180A and 2180B, and no notable changes were detected in the activities of the individual enzymes. Sporulation was accompanied by a rapid increase and then a decrease in total glucanase activity. Most of the increase was due to a dramatic rise in the activity of glucanase V, which appeared to be a sporulation-specific enzyme. Glucanase activity was not derepressed by lowering the glucose concentration in the growth medium.

Glucans containing (1→3)-β linkages are responsible for the strength and integrity of yeast cell walls, and it has been proposed that endogenous (1→3)-β-glucanases effect controlled wall hydrolysis during cell expansion, budding, conjugation, and sporulation (4). Evidence for this is based on reports that yeast (1→3)-β-glucanase activity varies throughout the life cycle. In *Saccharomyces cerevisiae*, budding and sporulation are accompanied by an increase in (1→3)-β-glucanase activity (1, 10, 11). Cells from conjugating cultures of *Hansenula wingei* and *Schizosaccharomyces versatilis* exhibit much higher (1→3)-β-glucanase activities than cells from nonconjugating cultures (2, 3).

Recent studies have shown that several different (1→3)-β-glucanases may occur in one yeast species, and these observations have raised the possibility that different enzymes may function at different stages during the cell cycle (4). With *S. cerevisiae* and *Schizosaccharomyces pombe*, for example, there is now some evidence to suggest that certain (1→3)-β-glucanases are specifically associated with sporulation (8, 11, 12). Direct association of a particular glucanase activity with a particular morphogenetic event, however, requires sound knowledge of the various glucanases that may occur in a yeast species and a means of measuring the individual enzymes at different stages during the cell cycle.

In the accompanying paper we describe the isolation and characterization of six different (1→3)-β-glucanases from cell-free extracts and cell walls of haploid and diploid strains of *S. cerevisiae* 2180 (6). These enzymes were designated glucanases I, II, IIIA, IIIB, IV, and V. We also report a convenient column chromatographic procedure for the separation and measurement of these individual enzyme activities (6). As part of an investigation into the function and regulation of β-glucanases in yeasts, in this paper we describe the variation of the six β-glucanases in *S. cerevisiae* during the vegetative growth cycle and during the sexual phases of conjugation and sporulation. Since the glucose concentration in the growth medium is known to regulate the production of β-glucanases in some fungi (9), the effect of this sugar upon (1→3)-β-glucanase production in *S. cerevisiae* is also described.

MATERIALS AND METHODS

Yeast strains. Haploid mating type strains *S. cerevisiae* 2180A (mating type a) and *S. cerevisiae* 2180B (mating type α) and diploid strain *S. cerevisiae* 595 are

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the same strains described in the accompanying paper (6). Strain 2180B was used in vegetative growth studies, and strains 2180A and 2180B were used for conjugation experiments. *S. cerevisiae* 595 was selected for sporulation studies due to its high sporulating ability.

Culture conditions for vegetative growth. Stock cultures were maintained on slants of malt extract agar and were subcultured into seed cultures containing YEG medium (5% glucose, 0.5% yeast extract) 48 h before an experiment was started. Liquid cultures for glucanase studies were grown in 3-liter flasks containing 1 liter of YEG medium. A 1% inoculum was used, and the cultures were incubated with orbital shaking (800 rpm) at 30°C. At different times after inoculation, the yeast cells were harvested by centrifugation, and cell walls, cell extracts, and wall autolysates were prepared as described in the accompanying paper (6).

To investigate the effect of glucose concentration in the growth medium on glucanase production, exponential phase cells were harvested, washed once with sterile distilled water and once with glucose-deficient (GD) medium (0.01% glucose, 0.5% yeast extract), and then suspended in the same medium. The culture was incubated, and samples of cells were harvested at regular intervals for glucanase analysis. After incubation for 25 h, the remaining cells were harvested, washed once with sterile distilled water and once with fresh YEG medium, and suspended in YEG medium. The cells were incubated for an additional 10 h, and samples were taken at regular intervals to check for renewed growth and levels of glucanase activity. Cell concentrations were estimated by measuring the optical densities of the cell suspensions at 550 nm. The glucose concentration in the growth medium was determined by using glucose hexokinase reagents obtained from Boehringer Mannheim Diagnostica.

Yeast conjugation. *S. cerevisiae* 2180A and 2180B were conjugated under the conditions for mass hybridization of *S. cerevisiae* described by Fowell (5). The two opposite mating type strains were grown separately in YEG medium to early stationary phase, harvested by centrifugation at a low speed, and suspended together in a glucose-enriched medium (10% glucose, 0.5% yeast extract). The cultures were aerated by shaking for 2 h at 20°C. The cell mixtures were compacted by low-speed centrifugation and left standing in the form of cell pellets in the centrifuge tube for 4 h at 20°C. During this period the cells underwent conjugation and zygote formation. Samples for analyses of glucanase activity were taken immediately after the cells were mixed and after 4 h in the centrifuge tubes. The percentages of conjugation and zygote formation were estimated by microscopic examination.

Yeast sporulation. The conditions used for synchronous sporulation of *S. cerevisiae* 595 at high cell concentrations were adapted from the method of Peterson et al. (7). Yeast cells were grown in YEG medium to stationary phase until glucose was just exhausted (36 h). The cells were harvested, washed twice with liquid acetate medium (1% potassium acetate, 0.05% glucose, 0.1% yeast extract), and resuspended in 100 ml of the same medium in a 1-liter conical flask to give a concentration of 1.0×10^8 cells per ml. The cells were incubated at 25°C with vigorous orbital shaking (800 rpm), and samples were taken at regular intervals to examine the glucanases. The extent of spore development was followed by microscopic counting of samples in a Petroff-Hausser counting chamber (C. A. Hausser, City, State). Both the age of the presporulation culture and the cell concentration in the sporulation medium influenced the percentage of spores formed, and the conditions chosen were those that yielded maximum sporulation. More than 70% of the cells had sporulated after 96 h.

Chromatographic separation and assay of (1→3)-β-glucanase activities. The (1→3)-β-glucanase activities in cell extracts and in cell wall autolysates were separated into the individual glucanase I, II, IIIA, IIIB, IV, and V activities by a system of chromatography through columns of DEAE–Bio-Gel A, CM–Bio-Gel A, and HTP–Bio-Gel, as described in the accompanying paper (6). Briefly, cell extracts or cell wall autolysates were dialyzed against 0.01M succinate buffer (pH 5.0) and then fractionated into glucanases I, II, III, and IV by chromatograpy over DEAE–Bio-Gel A. The unadsorbed effluent was collected and examined as glucanase V on CM–Bio-Gel A. Fractions containing glucanase III were subsequently resolved into glucanases IIIA and IIIB by passage through a column of HTP–Bio-Gel. (1→3)-β-Glucanase assays on cell extracts, cell walls, or column fractions were performed by using laminarin as the substrate (6). The (1→3)-β-glucanase elution profiles from the various columns were plotted, and the total activities for the individual (1→3)-β-glucanase were measured by assaying the pooled fractions containing each of the separated enzymes.

RESULTS

Variation of the (1→3)-β-glucanases of *S. cerevisiae* 2180B during vegetative growth. Cells from 48-h stationary phase culture were inoculated into fresh YEG medium and cultured for up to 7 days. At intervals during growth, cell samples were removed to prepare cell extracts and cell walls. Figure 1 shows the variation in the total (1→3)-β-glucanase activity in these fractions, along with the cell density and the concentration of glucose in the medium. The glucanase activity in both fractions increased steadily during exponential growth (10 to 15 h) and, after reaching a maximum level at the end of exponential growth, gradually declined. Low levels of (1→3)-β-glucanase were still detected in cells after 7 days of culture. More than 80% of the cell population was actively budding during exponential growth, whereas in stationary phase cultures more than 90% of the cell population was unbudded.

Samples of cell extracts and wall autolysates prepared from cells harvested at different times during the growth cycle were fractionated into their individual glucanase components by column chromatography. Chromatographic profiles showing resolution and the relative proportions of the different glucanases are shown in Fig. 2. Data on the activities of the different glucanases are summarized in Table 1. In a 48-h stationary

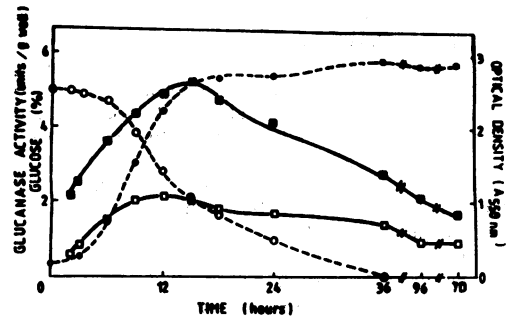

FIG. 1. Variation in (1→3)-β-glucanase activity in *S. cerevisiae* 2180B during vegetative growth. Symbols: ■, (1→3)-β-glucanase activity in cell extracts; □, (1→3)-β-glucanase activity associated with isolated cell walls; ●, cell density; ○, glucose concentration in culture medium. A_{550nm}, Absorbance at 550 nm.

phase culture (used as an inoculum for a fresh culture) glucanase IIIA predominated and accounted for more than 80% of the total glucanase activity in both extracts and wall autolysates. Small amounts of glucanases I, II, and V were present, and glucanases IIIB and IV were notably absent. As the culture entered the exponential phase, the proportion of glucanase IIIA activity decreased, and increasing amounts of glucanases I, II, and IIIB were found. The proportion of glucanase V activity progressively decreased during exponential growth.

By late log phase, glucanases IIIA and IIIB were of equal predominance in cell extracts, and each accounted for about 38% of the total activity. These were followed by glucanase II (15% of the total activity) and glucanase I (9%). Glucanase V accounted for only 4% of the total activity at this time, and glucanase IV was never detected in cell extract fractions.

In cell wall autolysates, glucanase IIIA activity decreased as exponential growth continued, and by late exponential growth it accounted for only 7% of the total activity. At this time glucanase IIIB was the predominant enzyme, accounting for 67% of the total activity. Glucanases II and I contributed 14 and 8%, respectively, of the total activity. Glucanase IV became detectable in the wall autolysates of exponential phase cells but at most accounted for only 0.1% of the total activity, whereas glucanase V activity dropped to 3% of the total activity.

As the cells entered the stationary phase, glucanase IIIA once again became the predominant enzyme in both cell fractions. This was mainly at the expense of glucanase IIIB and, to lesser extents, glucanases II and I, whose activities also decreased. Glucanase V activity in the cell wall fractions showed a significant increase and accounted for about 11% of the total activity (Table 1).

It is important to distinguish between the relative proportions of the individual enzymes and the absolute activity levels, as shown in Table 1. Of the six glucanases, the activity of glucanase IIIB showed the greatest variation. In both cell extracts and cell wall autolysates, this enzyme activity increased from virtually zero in resting cells to a maximal level during exponential growth and then decreased during the stationary phase. The actual level of glucanase IIIA activity in cell extracts remained almost constant despite the fact that its contribution to total activity decreased during exponential growth. However, the absolute values for glucanase IIIA activity in wall autolysates did decrease during exponential growth.

Variation in β-glucanases during conjugation. *S. cerevisiae* mating type strains 2180A and 2180B were mixed and incubated under conjugating conditions. After 2 h most of the cells had

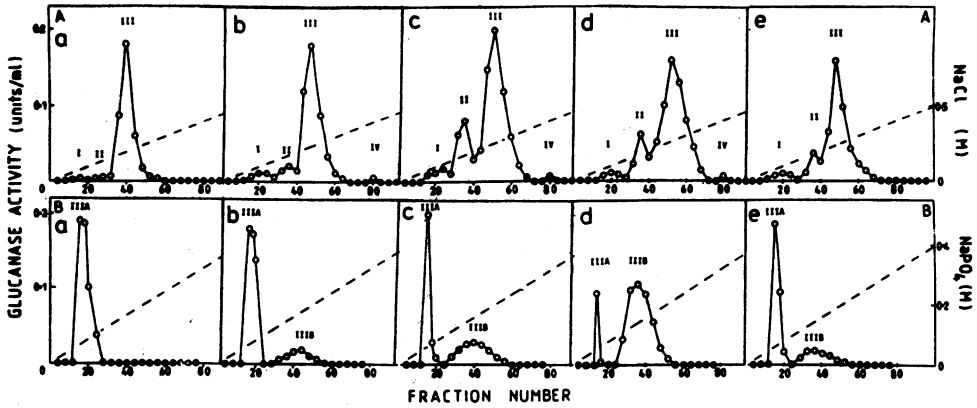

FIG. 2. (1→3)-β-glucanase profiles of cell wall autolysates of *S. cerevisiae* 2180B at stages during vegetative growth. (A) Profiles of glucanases I, II, III, and IV on DEAE–Bio-Gel. (B) Resolution of glucanases IIIA and IIIB on HTP–Bio-gel. (a) 48-h inoculum culture. (b) 6-h culture. (c) 12-h culture. (d) 20-h culture. (e) 36-h culture.

TABLE 1. Variation in the activities and proportions of individual (1→3)-β-glucanases in *S. cerevisiae* 2180B during vegetative growth

Prepn	Enzyme	Stationary phase (48 h)		Early log phase (6 h)		Mid-log phase (12 h)		Late log phase (20 h)		Stationary phase (36 h)	
		Activity (U/g of walls)[a]	% of total[b]	Activity (U/g of walls)	% of total	Activity (U/g of walls)	% of total	Activity (U/g of walls)	% of total	Activity (U/g of walls)	% of total
Cell extracts	Total activity	1.96	100	3.58	100	5.20	100	4.87	100	2.39	100
	Glucanase I	0.04	2.0	0.29	8.1	0.57	11.0	0.44	9.0	0.14	5.9
	Glucanase II	0.07	3.6	0.61	17.0	0.65	12.5	0.71	14.6	0.43	18.0
	Glucanase IIIA	1.73	88.3	1.77	49.4	1.95	37.5	1.83	37.6	1.77	74.1
	Glucanase IIIB	0	0	0.91	25.4	2.03	39.0	1.90	39.0	0.05	2.1
	Glucanase IV	0	0	0	0	0	0	0	0	0	0
	Glucanse V	0.12	6.1	0.18	5.0	0.21	4.0	0.19	3.9	0.12	5.0
Cell walls[c]	Total activity	1.02	100	1.69	100	2.06	100	2.01	100	1.30	100
	Glucanase I	0.01	1.0	0.15	8.9	0.20	9.7	0.16	7.8	0.08	6.2
	Glucanase II	0.01	1.0	0.14	8.3	0.36	17.5	0.29	14.1	0.09	6.9
	Glucanase IIIA	0.83	81.4	0.91	53.8	0.52	25.2	0.14	6.8	0.68	52.3
	Glucanase IIIB	0	0	0.34	20.1	0.94	45.6	1.37	66.5	0.31	23.8
	Glucanase IV	0	0	0.001	0.06	0.002	0.1	0.002	0.1	0	0
	Glucanase V	0.17	16.7	0.15	8.9	0.06	2.9	0.06	2.9	0.14	10.8

[a] Glucanase activity is expressed as units per gram (dry weight) of cell walls.
[b] Percentage of total activity.
[c] Cell walls were subjected to 12 h of autohydrolysis before fractionation into individual glucanases.

agglutinated, and about 5% were actually conjugating or forming tubelike extensions. By 4 h about 30% of the cells had conjugated, and a few zygotes had appeared. Approximately 50% of the cells were conjugating after 8 h. The total glucanase activities in either cell extracts or cell walls did not show any substantial increases during conjugation, up to 8 h. The proportions of the individual enzymes before and after conjugation were also very similar and resembled those of early stationary phase cultures (Table 1, 36-h data). However, although glucanase I contributed only a very minor percentage to the total extract and wall activities, this enzyme almost doubled its activity during conjugation. The contribution of glucanase IIIA to the total activity increased by 5 to 10% during conjugation, and this small change appeared to be at the expense of glucanase IIIB. Overall, the small enzyme changes noted during conjugation were reminiscent of a culture moving further into the stationary phase. The chromatographic profiles of the β-glucanases before and after conjugation were essentially the same as the 36-h data for vegetative cultures.

Variation in β-glucanases during sporulation. The diploid strains obtained from *S. cerevisiae* 2180A and 2180B matings did not produce high yields of sporulating cells (less than 10%) when they were tested on a variety of liquid and solid sporulation media. On the other hand, *S. cerevisiae* 595 readily produced sporulating cells in amounts that could be examined for glucanase activity, and since the β-glucanase system of this yeast strain was the same as that of *S. cerevisiae* diploid strain 2180D (6), it was considered suitable for our studies.

Cells of *S. cerevisiae* 595 were incubated under sporulation conditions, and the total glucanase activities in cell extracts and cell walls were measured at intervals during incubation. Figure 3 shows the development of sporulating cells during culture and the associated variation in (1→3)-β-glucanase activity. Just before the appearance of spores (36 to 48 h), there was a sharp increase in total glucanase activity in the cell extracts, followed by a sharp decrease in this activity (48 to 60 h). Wall-associated glucanase activity also increased after 36 h, but this increase was more gradual than the increase for cell extracts and did not peak until 60 h. After 60 h, this activity also decreased; by this time only 25% of the cells showed the presence of ascospores. The percentage of sporulating cells continued to increase after this time, although the glucanase activities in cell extracts and cell walls had decreased. By 96 h, approximately 75% of the cell population had sporulated. This sequence of events, during which glucanase activity peaked first in the cytoplasm and then in the cell walls and then decreased well before a majority of the cells had formed spores, was very reproducible.

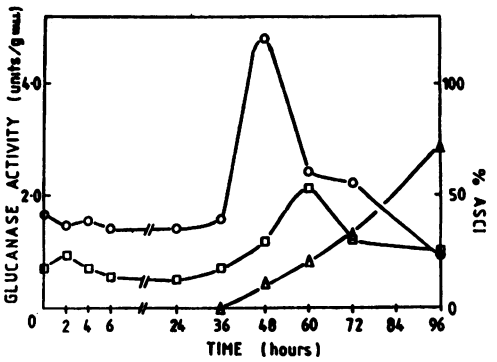

FIG. 3. Variation in total (1→3)-β-glucanase activities in cell extracts and cell walls of *S. cerevisiae* 595 during sporulation. Symbols: △, percentage of sporulating cells; ○, (1→3)-β-glucanase activity in extracts; □, (1→3)-β-glucanase activity in cell walls.

Table 2 shows the β-glucanase compositions of cells before transfer to sporulation medium and of cells after 48 or 60 h in the sporulation medium when the extract and wall-associated glucanase activities, respectively, had peaked. Before sporulation the cells exhibited a β-glucanase composition typical of early stationary phase cultures, when glucanase IIIA was the predominant enzyme (46% of the total activity), followed by glucanases IIIB (29%), II (12%), I (8%), and V (6%). After sporulation, the total glucanase level increased by almost threefold. However, with the exception of glucanase V, whose activity increased by about 30-fold, all of the other glucanases showed either no change or reductions in activity. Glucanase IIIB showed the greatest reduction. Glucanase V was by far the most prevalent enzyme in sporulating cells and accounted for more than 70% of the total glucanase activity in both cell extracts and wall autolysates. Glucanase IIIA was the next most prevalent enzyme, accounting for 20% of the total activity in both cell fractions. The contribution of glucanase IIIB decreased from around 28 to 30% to 1 to 2% after sporulation.

The glucanase V found after sporulation had the same properties on DEAE–Bio-Gel, CM–Bio-Gel, and Sephadex G-200 as the enzyme obtained before sporulation. Figure 4 shows the chromatographic resolution of glucanases I, II, IIIA, and IIIB of *S. cerevisiae* 595 before and after sporulation and the behavior of glucanase V (after sporulation) on CM–Bio-Gel and Sephadex G-200. These chromatographic properties of *S. cerevisiae* 595 glucanases are similar to those reported for *S. cerevisiae* 2180B (6).

In a control experiment, cells of haploid strain

TABLE 2. Variation in the activities and proportions of individual (1→3)-β-glucanases in *S. cerevisiae* 595 during sporulation in acetate medium

Prepn	Enzyme	Presporulation culture		Sporulation culture	
		Activity (U/g of walls)	% of total activity	Activity (U/g of walls)	% of total activity
Cell extracts	Total activity	1.44	100	3.26[a]	100
	Glucanase I	0.10	6.9	0.08	2.6
	Glucanase II	0.16	11.1	0.16	4.8
	Glucanase IIIA	0.67	46.5	0.61	18.7
	Glucanase IIIB	0.43	29.9	0.08	2.4
	Glucanase IV	0	0	0	0
	Glucanase V	0.08	5.6	2.34	71.4
Cell walls[b]	Total activity	0.67	100	1.79[c]	100
	Glucanase I	0.05	7.5	0.02	1.1
	Glucanase II	0.08	11.9	0.03	1.7
	Glucanase IIIA	0.31	46.2	0.36	20.1
	Glucanase IIIB	0.19	28.5	0.03	1.7
	Glucanase IV	0	0	0	0
	Glucanase V	0.04	5.9	1.35	75.4

[a] The glucanase composition of a 48-h culture was analyzed.
[b] Cell walls were subjected to 12 h of autohydrolysis before fractionation into the glucanase components.
[c] The glucanase composition of a 60-h culture was analyzed.

S. cerevisiae 2180B were incubated under sporulation conditions. There was no cell growth or ascospore formation under these conditions, as expected. Total glucanase activity did not increase during incubation, and the glucanase profiles before and after incubation were similar. Therefore, we conclude that glucanase V production is specifically due to sporulation and is not a result of changes in culture conditions.

Variation in β-glucanases under conditions of glucose starvation. When cells from exponential phase cultures were transferred to a glucose-deficient medium, growth stopped almost immediately, and the level of glucanase activity associated with the cell wall fraction decreased by 50% within 5 to 10 h in the new medium. In contrast, glucanase activity in cell extracts showed little change during this period, but had decreased by about 13% after 25 h. The composition of the wall-associated (1→3)-β-glucanase system was analyzed after 5 h in GD medium, and the activities of glucanases I, II, and IIIB were found to have decreased by 50, 50, and 65%, respectively, whereas glucanase IV completely disappeared (Table 1, 12-h data). However, the level of glucanase IIIA remained constant, and glucanase V activity increased by 27%. After 25 h, the cells were transferred from GD medium to fresh YEG medium. Growth and budding started after an initial lag phase, and wall-associated and extract activities increased to their original levels. After 10 h, this culture entered the stationary phase, and the levels of (1→3)-β-glucanase activities in cell extracts and wall preparations decreased in line with the observations presented in Table 1.

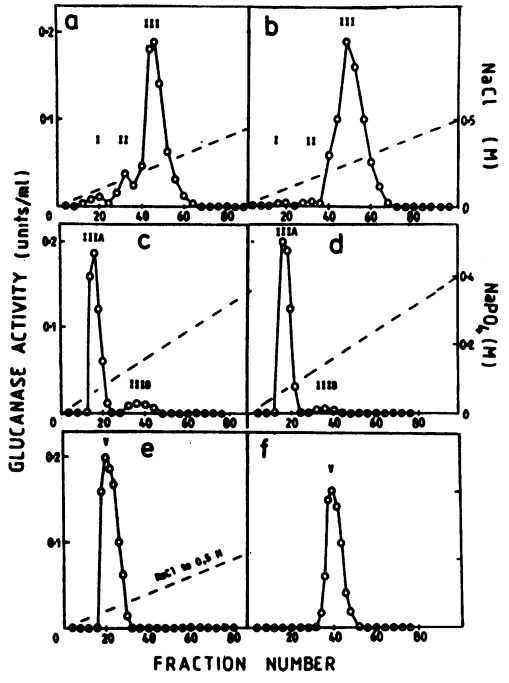

FIG. 4. (1→3)-β-glucanase profiles of cell wall autolysates of *S. cerevisiae* 595. (a and b) Glucanases I, II, and III on DEAE–Bio-Gel before and after sporulation, respectively. (c and d) Glucanases IIIA and IIIB on HTP–Bio-Gel before and after sporulation, respectively. (e and f) Glucanase V after sporulation on CM–Bio-Gel and Sephadex G-200, respectively. The elution conditions used are described in the accompanying paper (6).

DISCUSSION

In the accompanying paper (6) we report the occurrence of six different (1→3)-β-glucanases in *S. cerevisiae*. In this study we demonstrated that these enzymes have different functions and independent patterns of variation during the cell cycle.

The increases in extract and wall-associated (1→3)-β-glucanase activities during exponential growth and their decreases during the stationary phase are consistent with other reports (1, 10) and support the proposal that (1→3)-β-glucanases act as wall modifiers and plasticizers during cell expansion and budding.

Of the two exoglucanases, only glucanase II activity increases during exponential growth and appears to be involved in vegetative division. It may be significant that this exoglucanase can initiate action on glucan substrates at points other than the nonreducing terminal residue (6). Moreover, this enzyme seems to exhibit some synthetase or transferase activity in addition to its hydrolytic action (6), so that it may specifically function in cell wall expansion through the insertion of new glucosyl units. This interesting possibility certainly warrants further study. The activity of exoglucanase IIIA remains relatively constant or even decreases during exponential division and, therefore, appears to have no direct function at this stage of growth. This is consistent with a previous report that mutants lacking this enzyme exhibit normal growth and budding (13). However, the striking predominance of exoglucanase IIIA in stationary phase and older cultures suggests some functional role at this stage of the growth cycle.

Endoglucanases I, IIIB, and IV all show varying increases in activity during exponential growth and, consequently, may play important hydrolytic roles during cell wall expansion and budding. Specific definition of these roles requires further study. The behavior of glucanase IIIB is particularly noteworthy since this enzyme activity is completely absent in stationary or resting-phase cells but is rapidly synthesized or activated to become the predominant glucanase during exponential growth. Since glucanase IIIB hydrolyzes laminarin to produce mainly laminaripentaose (6), it is possible that this enzyme randomly cleaves cell wall glucans at widespread points to allow insertion of new glucosyl units as needed for wall expansion. The decreasing activity of endoglucanase V during exponential growth suggests that this enzyme is not required for vegetative division.

It was surprising to find little alteration in total glucanase activity or in individual glucanase composition during cell conjugation, when considerable localized wall expansion and dissolution are expected to occur (2). These findings are consistent with reports by Rey et al. (10) but are contrary to the increased glucanase activities noted during the conjugation of *H. wingei* (2) and *Schizosaccharomyces* species (3, 8). Thus, the role of glucanases in yeast conjugation seems to be more complicated than originally thought and demands closer examination.

Sporulation in *S. cerevisiae* was accompanied by a large increase in total (1→3)-β-glucanase activity, and this was mainly due to a dramatic (30-fold) increase in the activity of glucanase V. This enzyme accounted for around 75% of the total glucanase activity in sporulating cultures, and our data strongly indicate that this is a sporulation-specific enzyme. It is not possible to state the exact role of this endoglucanase in the sporulation process, but since its development occurs largely before the majority of ascospores have actually formed, it may function in the mobilization and organization of vegetative cell walls into asci and ascospore walls. Precedents for such glucanase function have been reported previously for *Aspergillus nidulans* (14). It is unlikely that glucanase V is involved in the lysis of ascus walls to release ascospores since the spores were still encased within the asci long after the level of glucanase V activity had increased and then decreased. Glucanase activity has been implicated in ascus lysis for some other yeast species (4). We found no evidence for the involvement of exoglucanase activity in the sporulation of *S. cerevisiae* as recently described by Rey et al. (12).

The production (1→3)-β-glucanases by some fungi is repressed by the presence of glucose in the culture medium and is derepressed under conditions of glucose limitation (9). Repression of glucanase activity by glucose was not observed for *S. cerevisiae*, and, in fact, glucose limitation had the effect of switching off exponential growth and causing a reduction in the levels of those glucanases (namely, glucanases I, II, IIIB, and IV) suspected of acting during vegetative division. Glucose limitation did cause a slight increase in glucanase V activity, and this might be interpreted as a response in preparation for sporulation, which in *S. cerevisiae* is encouraged by conditions of glucose deprivation.

It is pertinent to point out certain limitations associated with the measurement of glucanase activity when laminarin is used as the substrate and reducing sugar formation is used as an index of activity. First, an enzyme that produces large amounts of glucose (e.g., glucanase IIIA) or low-molecular-weight oligosaccharides appears to be more active than an enzyme which produces large products (e.g., laminaripentaose production by glucanase IIIB). Thus, for example, glucanase IIIA appears to be more active

than glucanase IIIB when, in reality, there could be more molecules of the latter. Second, one glucanase may have totally different levels of activity on different (1→3)-β-glucan substrates. For example, although glucanase I activity appeared to be a relatively minor component of total glucanase complex activity (Table 1), this conclusion was based on the use of laminarin as the substrate. This might not have been the case if a more natural substrate (e.g., a wall glucan) had been used. However, the use of laminarin as the substrate did permit valid comparisons of the same enzyme at different stages during the life cycle.

Regulation or control of glucanase activity was not considered in this study. Here and in the accompanying paper (6), we show that the (1→3)-β-glucanase system of *S. cerevisiae* is far more complex than previously thought and that the individual enzymes of the system vary independently throughout the life cycle. It is not known whether these variations are due to enzyme synthesis or enzyme activation or both, and these questions, along with questions concerning possible control mechanisms, remain open for further investigation.

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