Reproducible and Rapid Methods for the Isolation and Assay of a-Factor, a Yeast Mating Hormone

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The *Saccharomyces cerevisiae* mating hormone a-factor has been difficult to isolate reproducibly in sufficient yields by methods using ion-exchange chromatography, probably because of its pronounced hydrophobicity. In this work, a hydrophobic adsorbent (Amberlite XAD-2), in an insoluble bead form, was used to isolate larger (up to sixfold greater than previous reports) and quite reproducible (12% standard deviation) quantities of a-factor by adsorption from cell-free filtrates of a cultures. Moreover, when the beads were added to the cultures at the time of inoculation, sixfold greater yields were obtained than when a-factor was adsorbed to the beads from cell-free filtrates. a-Factor was readily eluted from the beads with 1-propanol. The same adsorbent could also be used in the partial purification of the less hydrophobic α -factor. Adsorption of both hormones by Amberlite XAD-2 gave a degree of purification comparable to that obtained by the first steps of previously published methods while providing larger yields of hormones. The present procedure is shorter, simpler, and, for a-factor, more reproducible. The activities of both hormones were quantitated by using an assay in which the size distribution of cells in the population was monitored after the addition of hormone of the opposite mating type. The extent of increase in cell size which accompanies hormone treatment is a function of the hormone concentration. To ensure solubilization of a-factor in the aqueous bioassay system, samples were diluted into bovine serum albumin solutions and sonicated before assaying. The resulting assay is most sensitive at hormone concentrations between 0.05 and 2 U/ml, can reliably detect as little as 0.16 ng of hormone, gave results reproducible within 16%, and is convenient for a large number (>100) of samples.

Sexual conjugation in the yeast *Saccharomyces cerevisiae* requires in part the action of two diffusible peptide mating hormones, a-factor and α -factor. Haploid mating-type a cells constitutively secrete a-factor that acts on α cells, and α cells produce α -factor, which is effective on a cells (3, 12, 30). Cells that are incubated with the hormone isolated from cultures of the opposite mating type (i.e., heterologous hormone) exhibit several characteristic responses that are also seen in normal mating mixtures of the two cell types. These include cell division arrest leading to the accumulation of unbudded cells in the G1 phase of the cell cycle (3, 5, 16, 30), the induction or enhancement of heterosexual agglutinability (2, 14, 31), elongation of the cells to pear-shaped forms called "shmoos," which can be seen after one generation time of division arrest (3, 12, 23), and enhanced binding of fluorescent-

ly labeled concanavalin A to the elongated region of shmoos (28), thus demonstrating the localization of changes in cell wall polysaccharides that have been reported for a shmoos (21). The involvement of the hormones in conjugation is also indicated by the properties of mutants isolated for their inability to mate (23) or for their resistance to α -factor (17, 24).

Both mating hormones have been purified to homogeneity (1, 13, 26), and α -factor has been sequenced (27) and synthesized chemically (8, 25). Studies on the cellular response to hormones and the cell-hormone interaction have usually employed α -factor (4, 5, 15-17, 20, 21). Although the amino acid composition of a-factor has been reported (1), it has been quite difficult to isolate this hormone reproducibly or in sufficient yields relative to contaminating materials to assay it reliably and sensitively (1, 3; J. R. Strazdis, Ph.D. thesis, Rutgers University, New Brunswick, N.J., 1980). The amino acid composition, H₂N-Tyr-(Asx₁, Gly₁, Ala₁, Val₁, Ile₂, Phe₁, Lys₁, Trp₁, Pro₁) (1), suggests that at least

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part of this difficulty can be attributed to the hydrophobicity of the molecule. The ion-exchange chromatography and aqueous conditions used in the initial steps of the published purification and assay methods (1, 3) may not allow complete or reproducible recovery or detection of the hormone. Because shmoo formation is a mating-type specific cellular response elicited by the mating hormones and is readily observed by microscopic examination of assay cells, such morphological changes have been widely used to quantitate both *a*-factor and α -factor (1, 3, 12, 23). The definition of 1 U/ml in this assay is the lowest dilution of a hormone sample that will give shmoo formation (3, 12); therefore, by definition, this assay cannot be used to detect hormone concentrations of less than 1 U/ml. Furthermore, it involves some subjective judgment on the part of the investigator to distinguish between a shmoo and an oddly shaped nonresponding cell, particularly at hormone concentrations around 1 U/ml. More recent assay methods involve the correlation of hormone concentration with physiological events other than shmoo formation, such as the inhibition of cell division (3, 5, 30) or the induction of agglutinability (2, 14, 31). In general, these are not suitable for large numbers of samples or, in some cases, low concentrations of hormones. In this paper, we describe a new assay that is based on the increase in cell size which occurs when haploid cells are incubated with heterologous mating hormone. The assay is sensitive, reliable, and convenient for numerous samples. By using this assay, we have developed new methods for the rapid and reproducible isolation of *a*-factor (and α -factor) with a hydrophobic adsorbent. Therefore, it is now possible and easy to obtain large quantities of partially purified *a*-factor.

MATERIALS AND METHODS

Yeast strains and culture conditions. The isogenic haploid wild-type strains X2180-1A (mating type *a*) and X2180-1B (mating type α) were used in this study and are available from the Yeast Genetics Stock Center (Berkeley, Calif.). The culture medium in all experiments was a minimal glucose (MV) medium (30) containing 0.67% yeast nitrogen base without amino acids (Difco Laboratories, Detroit, Mich.) and 2% glucose. All cultures were incubated at 30°C with aeration. For routine culturing and stock maintenance, YEPD medium (1% yeast extract, 2% peptone, 2% glucose, and 2% agar) was used.

Determination of hormone activity. Several assay procedures with different sensitivities and ranges were used to determine *a*-factor activity.

(i) **Morphogenetic assay.** High concentrations of *a*-factor were assayed by looking for morphological changes in α cells (3). Assays were performed in MV medium containing 0.05 M NaPO₄ (pH 5.0), 0.2 mg of bovine serum albumin (BSA; A grade; Calbiochem, La Jolla, Calif.) per ml, and 1.33×10^6 exponentially

growing α cells per ml, in a final volume of 0.375 ml. Assay mixtures were sonicated for 5 min in an ultrasonic cleaning bath before the addition of α assay cells which had been prewashed with water. If prior dilution of *a*-factor was required, dilution was made into BSA solution (minimum BSA concentration, 0.2 mg/ml), and the solution was sonicated for 5 min after each dilution. The assay cultures were incubated with aeration at 30°C for 4 to 5 h and examined microscopically for shmoo. Twofold serial dilutions of active samples were tested to determine the lowest concentration causing detectable shmoo formation. This concentration (corresponding to about 20% shmoo at 4 h) has been defined as 1 U of activity per ml (3). The assay is sensitive at *a*-factor concentrations of 1 U/ml or higher.

(ii) **Cell division inhibition assay.** Lower concentrations of *a*-factor were assayed by following the transient inhibition of cell division in exponentially growing cultures of α cells (3, 30). Assay volumes were 5.0 ml and contained the same concentrations of components as in the morphogenetic assay. The cultures were incubated with aeration at 30°C, and 0.2-ml samples were removed at hourly intervals for 7 to 10 h. After sonication, the cell numbers in these samples were determined in a model Z_F Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.), as described previously (4). The length of time that cell division is inhibited is linearly proportional to the logarithm of the amount of *a*-factor added (3). One unit of *a*-factor activity per ml, determined by the morphogenetic assay produces a period of inhibition approximately equal to one generation time. Because of sampling limitations, the assay is sensitive at *a*-factor concentrations of 0.5 U/ml or higher, and the number of samples which can be assayed at one time is limited to about 15.

(iii) **Cell volume change assay.** The cell volume change assay method presented here was developed to assay very low concentrations of *a*-factor. Samples were assayed by measuring the increases in size or volume which occur in α cells that have been incubated with *a*-factor. The assay mixtures used were the same (0.375 ml) as those described above for the morphogenetic assay. The assay cultures were incubated with aeration at 30°C for 3 h, at which time 0.2-ml samples were removed for analysis (after sonication) with a Coulter Counter to determine the size distributions of cells in the samples. The size increase observed in an α cell population is a function of the amount of *a*-factor added. After 3 h of incubation, 1 U of activity per ml (determined by morphogenetic assay) produces an increase in the overall cell size in the population such that 77% of the cells are larger than the median of the control population (see Results). This correlation provides a more precise redefinition of 1 U of hormone activity, as described in Results. The assay is sensitive at concentrations of *a*-factor from 0.05 to approximately 2 U/ml.

The three assay procedures described above for *a*-factor were also used in the determination of α -factor activity on *a* cells, with the omission of BSA and sonication, which were not required because α -factor is quite soluble in the aqueous assay system. In all assays for either hormone, control cells were incubated with an equal concentration of the solvent for the hormone.

α -Factor preparation. Mating-type α cells were inoculated at a density of 10^6 cells per ml and grown aerobically for 48 h at 30°C on a rotary shaker at approximately 200 rpm or in a 40-liter fermentor that was aerated with an agitation speed of 300 rpm and an air flow of 20 liters/min. The cells were removed by centrifugation, and α -factor was partially purified from the culture filtrate by chromatography on Amberlite CG-50, as described by Duntze et al. (13) or as modified by Lipke and Ballou (20); this was followed by methanol extraction (13).

α -Factor preparation. Mating-type α cells were inoculated at a density of 10^7 cells per ml and grown for 24 h at 30°C on a rotary shaker at approximately 200 rpm or in a 40-liter fermentor that was aerated with an agitation speed of 300 rpm and an air flow of 20 liter/min. The cells were removed by centrifugation, and α -factor was partially purified by hollow-fiber ultrafiltration with an Amicon H1DP-10 hollow-fiber membrane cartridge and by chromatography on SP-Sephadex C-25 or phosphocellulose (3). Additional purification methods are described in Results.

Preparation of Amberlite XAD-2 resin. For α -factor preparations, the polystyrene resin Amberlite XAD-2 (Rohm and Haas, Philadelphia, Pa.) was first washed with excess distilled water, then packed into a 40°C temperature-jacketed column. The column was washed with 6 column volumes of 1,2-dichloropropane-1-propanol (1:3) and 6 column volumes of 1-propanol at a flow rate of 1 to 2 volumes/h. The resin was stored in propanol, washed with excess distilled water, and sterilized by autoclaving in distilled water before use. The resin was never allowed to run dry when aqueous solutions were used.

For α -factor preparations, the resin was washed in the same way, with the exception that methylene chloride-methanol (1:3) and methanol were used in place of 1,2-dichloropropane-1-propanol and 1-propanol (32).

Determination of protein and carbohydrate. Protein was determined by the procedure of Lowry et al. (22), with BSA as the standard. Total carbohydrate was determined by the phenol-sulfuric acid method of Dubois et al. (10, 11), as described by Hodge and Hofreiter (18), with glucose and mannose as standards.

All other chemicals used in this study were reagent grade and were purchased from standard suppliers.

RESULTS

Cell volume change assay. We have used a Coulter Counter and Channelyzer to determine the size distribution and mean volume of cells in a haploid yeast population which had been incubated with heterologous hormone. Treatment of exponentially growing cells with 6 U of the hormone per ml (determined by morphogenetic assay) for 2 h (one generation time under these conditions) resulted in a 1.6-fold increase in the mean volume of the population. This increase in volume is consistent with the reports that hormone treatment causes cells to arrest in the G1 phase of the cell cycle, so that cell division and DNA synthesis do not occur, but synthesis of protein and RNA continues (4, 16). At lower

hormone concentrations, the increase in the mean volume of a population was found to be a function of the concentration of hormone with which the cells had been treated. The size distributions of α cells which had been treated with increasing α -factor concentrations are shown in Fig. 1. As α -factor concentration increased, the distributions shifted to the right and broadened, showing that mean cell size did indeed increase as a result of increasing hormone concentration. Similar curves were obtained by treating α cells with increasing amounts of α -factor (data not shown).

This dose-response relationship could be simply quantitated by determining the median size of the control culture (no hormone treatment) with the Channelyzer and determining the percentage of cells in each assay sample which were larger than the median of the control culture. For the control cells, the percentage of cells larger than the median size was approximately 50% (see the legend to Fig. 2), whereas for hormone-treated cells it ranged from about 50 to

FIG. 1. Size distributions of α cells incubated with α -factor. Exponentially growing α cells were treated for 4 h with increasing concentrations of α -factor (prepared by SP-Sephadex and assayed by morphogenetic assay). The samples were diluted into isotonic counting solution, and the number of cells in a given size range was determined by performing counts on each sample at various lower threshold settings with a Coulter Counter. The data generated manually in this way approximate data obtained automatically with a Channelyzer. However, since a limited number of data points are used to generate idealized curves here, they cannot be quantitatively compared with the Channelyzer results in Fig. 2. Symbols: $\circ$, control, no addition; $\bullet$, 0.5 U/ml; $\square$, 1 U/ml; $\blacksquare$, 5 U/ml.

FIG. 2. Size increase in α cells incubated with α -factor. Exponentially growing α cells were treated for 4 h with increasing concentrations of α -factor (prepared by SP-Sephadex and assayed by morphogenetic assay). Samples were analyzed with a Coulter Channelyzer to determine the percentage of cells larger than the median of the control. Because the Channelyzer approximates a continuous cell size distribution with 99 discrete channels and the median may not coincide exactly with a channel, the channel closest to the true median was used. Therefore, the percentage of cells in the control population larger than the size represented by this median channel varies slightly (ca. 50%).

nearly 100% after 4 h of incubation. The percentage of cells in a population larger than the median size of the control was a function of the hormone concentration with which the population has been treated (Fig. 2). A good correlation is evident from these data relating α -factor concentration and percentage of cells larger than the control median. The percentage of cells in the control culture that were larger than the median (approximately 50%) was then subtracted from all samples to determine "percent response." Thus, percent response in control cultures without α -factor is 0%, and hormone-treated cultures show percent response values ranging from 0 to 50%.

The percent response values obtained with the cell volume change assay were correlated with units based on the morphogenetic assay (3, 26), and 1 U of α -factor per ml caused a 27% response after 3 h of incubation. (In Fig. 1 and 2, 4-h incubation periods were used.) At low hormone concentrations (less than 1 U/ml in Fig. 3), percent response was linearly proportional to the log of the hormone concentration with which cells had been treated, and the assay was sensitive and reliable at hormone concentrations as low as 0.1 U/ml in Fig. 3. Other experiments show that we can routinely detect concentrations as low as 0.05 U/ml, and the upper limit (1 to 4 U/ml) is dependent on the purity of the hormone preparation. Comparable results were obtained for α -factor with a assay cells (data not

FIG. 3. Percent response as a function of α -factor concentration. Exponentially growing α cells were treated with increasing concentrations of α -factor (prepared by Amberlite XAD-2) for 3 h, and samples were analyzed with a Channelyzer to determine percent response. Dilutions of α -factor were made into BSA with sonication. The error bars represent 1 standard deviation of duplicate determinations.

shown). Therefore, we redefined 1 U/ml as that concentration of mating hormone that elicits a 27% response in cells of the opposite mating type after 3 h of incubation in the cell volume change assay described above. This definition is used in all of the experiments reported below.

Nonspecific effects on cell size by mating factor preparations were determined by treating α cells with **a**-factor preparations and α cultures with α -factor preparations. With the exception of unfractionated culture filtrates which had been concentrated 10-fold by rotary evaporation (in which the concentration of salt and other material is high), the homologous preparations did not affect cell size.

Repeated assay of any given α -factor sample gave very reproducible estimates of activity; however, such reproducibility was not initially found for **a**-factor. This variability with **a**-factor samples was probably due to difficulty in solubilizing reproducibly the hydrophobic **a**-factor under aqueous assay conditions (Strazdis, thesis). Attempted solubilization with detergents, including Triton X-100, Tween 20, Tween 60, Tween 80, and octyl- β -D-glucopyranose, was unsuccessful; none of these, except octyl- β -D-glucopyranose, increased activity or reproducibility (data not shown). Although the addition of octyl- β -D-glucopyranose frequently enhanced activity substantially, this result was not always obtained, even with the same stocks of the detergent and **a**-factor. The detergent also significantly increased variability (by as much as 100%) among multiple assays at the same concentration of an **a**-factor stock (data not shown). The possibility that the enhancement of **a**-factor activity by octyl- β -D-glucopyranose was due to trace contamination by octanol (9, 19) was tested by adding small quantities of octanol and several other higher alcohols to the assays to solubilize the hormone. These provided little or no increase in **a**-factor activity while causing greater variability in duplicate assays (data not shown). In contrast, the presence of at least 0.2 mg of BSA per ml in all aqueous solutions and sonication of samples after each dilution of **a**-factor in aqueous solution led to excellent agreement between duplicate samples (Fig. 3) and gave results reproducible within 16% when the same **a**-factor sample was assayed in four independent experiments (Table 1). Occasionally, sonication in the presence of BSA increased activity slightly in addition to reducing variability.

Partial purification of **a-factor.** The elucidation of the amino acid composition of **a**-factor (1) revealed that the peptide is rich in hydrophobic amino acids. Therefore, partial purification by hydrophobic adsorption might lead to higher and more reproducible yields of **a**-factor than ad-

sorption to ion-exchange resins. Amberlite XAD-2, a polystyrene resin often used in adsorption or organic material from aqueous solutions, is manufactured in the form of insoluble beads which can be used in columns or in batch adsorption. The beads were added to cell-free filtrate from an α culture (100 ml of prewashed beads per liter of culture filtrate) and incubated for 24 h at 30°C with shaking. The beads were subsequently packed into a 40°C constant-temperature column, and the column was washed first with water and then with 40% methanol (3 column volumes of each, with a flow rate of 1 to 2 volumes/h). Activity was eluted from the column with 3 column volumes of 1-propanol, and fractions were collected. Active fractions were pooled, taken to dryness by rotary evaporation, and suspended in a small volume (1 to 2 ml) of dimethyl sulfoxide. A typical elution profile is shown in Fig. 4. The yields of **a**-factor (typically 800 U/liter of culture filtrate) were higher than those achieved with Amicon ultrafiltration and SP-Sephadex C-25 chromatography, which varied from 8 to 480 U/liter of culture filtrate in five different preparations (Strazdis, thesis), and less than 2% of the initial **a**-factor activity in the culture filtrate remained unadsorbed. Adsorption from culture filtrates (adjusted to pH 5.0 with 1 M NaOH) and adsorption at pH 2.6 (no adjustment) yielded the same quantities of **a**-factor. Once adsorption to the beads was carried out and they were briefly rinsed with water, it was possible to store them in water at -20°C for more than 1 month before eluting **a**-factor without any loss of activity. Equivalent amounts of **a**-factor were obtained by batchwise elution in flasks, although constant-temperature elution in columns achieved a higher degree of purification (Strazdis, thesis; unpublished data).

In addition to carrying out adsorption from cell-free filtrates of α cultures, Amberlite XAD-2 beads could be added directly to the cell cultures at the beginning of growth, allowing adsorption to occur throughout the 24-h period of cell growth. In addition to shortening the preparation time, this approach yielded larger quantities of **a**-factor. When beads were added to the

TABLE 1. Reproducibility of **a**-factor assays in the presence of BSA with sonication

Expt	a -Factor (U/ml)
1	6,700
2	7,800
3	5,360
4	6,080
Mean	6,480 $\pm$ 1,030 ^a

^a One standard deviation from the mean.

FIG. 4. Propanol elution of *a*-factor from Amberlite XAD-2. Amberlite XAD-2 beads (5 ml of prewashed beads in water) were added to 50 ml of cell-free filtrate from a culture and incubated for 24 h at 30°C with shaking. The beads were packed into a constant-temperature column at 40°C and washed first with 15 ml of water and then with 15 ml of 40% methanol. Fractions (1.5 ml each) were collected, and activity was eluted with 30 ml of 1-propanol.

culture at the beginning of growth, 1 liter of culture yielded 5,100 U of *a*-factor activity (versus 800 U by adsorption from cell-free filtrates). The separation of beads from yeast cells before elution of *a*-factor was easily achieved by rinsing them with water in a flask, since the beads are large and settle to the bottom much more rapidly than do yeast cells. The beads were then packed into a column, and *a*-factor was eluted as described above.

The degree of purification obtained with Amberlite XAD-2 was assessed by quantitating the hormone activity and the carbohydrate and protein content of an *a*-factor sample that was partially purified by elution from Amberlite XAD-2 beads. Activity was adsorbed from a 50-ml *a* cell culture during growth, the yeast cells were removed by rinsing with water, column elution was performed at 40°C, and 1.5-ml fractions were collected. Active fractions were combined, and the pool was assayed for *a*-factor activity with the cell volume change assay and for protein and carbohydrate content. The results are compared with the results reported for SP-Sephadex C-25 elution followed by Amicon

TABLE 2. Partial purification of *a*-factor by Amberlite XAD-2 and SP-sephadex

Purification method	Total activity (U/liter of culture)	Total carbohydrate (mg)	Total protein (mg)	Sp act (U/mg of protein)
Amberlite XAD-2 elution	5,100	1.8	14	370
Amicon concn and Sp-Sephadex elution	120 ^a –1,900 ^b	0.4 ^a	1 ^a	120 ^a

^a Betz et al. (3).

^b Betz and Duntze (1).

concentration in Table 2 (1, 3). The values reported in Table 2 represent the yields normalized to 1 liter of a culture. With Amberlite XAD-2, the total amount of protein and carbohydrate recovered in the preparation was greater, although the yield of *a*-factor was also much larger. Therefore, the overall degree of purification obtained in this step was somewhat better than that reported for Amicon concentration and SP-Sephadex elution.

Finally, the yields of *a*-factor from Amberlite XAD-2 partial purification have been quite reproducible. The results from six different preparations are shown in Table 3; yields were typically 800 U of *a*-factor from 1 liter of cell-free filtrate (in cases in which adsorption was carried out after growth of the culture), with a standard deviation of 12%. Table 3 also illustrates the amenability of this method to modification; variations in *a*-factor concentration and in pH during adsorption and variations in method employed

TABLE 3. Yields of *a*-factor obtained by partial purification with Amberlite XAD-2

Source of <i>a</i> -factor	pH during adsorption	Method of elution	Original culture volume (liters)	Total units of <i>a</i> -factor	Units of <i>a</i> -factor per liter
Amicon concentrate	2.6	Column	2.4	1,570	654
Amicon concentrate	2.6	Column	1.2	1,000	833
Amicon concentrate	2.6	Column	1.2	1,000	833
Culture filtrate	5.0	Column	1.0	750	750
Culture filtrate	5.0	Batch	0.1	94	940
Culture filtrate	5.0	Batch	4.0	3,200	800

FIG. 5. Methanol elution of α -factor from Amberlite XAD-2. Amberlite XAD-2 beads (12 ml of prewashed beads in water) were added to 120 ml of cell-free filtrate from α cultures and incubated for 24 h at 30°C with shaking. The beads were packed into a constant-temperature column at 40°C and washed first with 36 ml of water and then with 36 ml of 40% methanol. Fractions (3 ml each) were collected, and activity was eluted with 90 ml of 80% methanol. Subsequent washing of the column with 100% methanol eluted additional material absorbing at 280 nm, but this material did not have α -factor activity.

for elution did not change the yield of α -factor significantly. The α -factor activity recovered from Amberlite XAD-2 after adsorption from either cell-free culture filtrates or growing cultures exhibited all of the activities reported to be associated with α -factor, including G1 arrest, induction of agglutinability, morphological changes to the shmoo form, and asymmetric binding of fluorescently labeled concanavalin A (data not shown).

Partial purification of α -factor. Adsorption to Amberlite XAD-2 was also used in the partial purification of α -factor. Although α -factor is not as hydrophobic as α -factor, it contains a relatively large proportion of hydrophobic amino acids and was readily adsorbed to the resin. Adsorption was carried out from cell-free filtrates of α cultures under the same conditions described for α -factor and was followed by washing the column (at 40°C) first with 3 column volumes of water and then with 3 column volumes of 40%

methanol. Elution of α -factor was more rapid than that of α -factor under the same conditions, and 80% methanol was sufficient to elute the activity in a relatively sharp peak (Fig. 5). The yield of α -factor obtained with this method was 4,200 U/liter of culture filtrate, the same as that reported by Duntze et al. (13). The degree of purification (specific activity of 380 U/mg of protein) was comparable to that obtained for α -factor with Amberlite XAD-2.

The addition of Amberlite XAD-2 beads to α cultures for adsorption of the hormone during growth yielded about twofold more α -factor than did adsorption from culture filtrates; the yield was 7,500 U/liter of culture. As with α -factor, once α -factor had been adsorbed to Amberlite XAD-2 and the beads had been rinsed with water, it was possible to store beads in water at -20°C before elution without loss of activity, and batchwise elution yielded the same quantities of α -factor as did elution from columns. As described above for α -factor, the α -factor obtained by adsorption to Amberlite XAD-2 possessed all of the activities attributed to the hormone.

DISCUSSION

The methods reported in this paper for the rapid and reproducible isolation and assay of α -factor, a hydrophobic peptide, should now make it possible to analyze the action of this hormone on α cells and to use α -factor in studies of the yeast cell cycle and of cellular regulation by the mating-type locus. Previous attempts to work with α -factor have been generally unsuccessful, probably because its hydrophobicity prevents its complete solubilization under the conditions used. Moreover, the published assays were not sensitive or convenient enough for the comparison of α -factor yields from different strains under different conditions.

The cell volume change assay described herein is based on the G1 arrest induced by mating hormones in sensitive cells (3, 4, 16, 29). Although the cell division cycle and DNA replication are blocked, the arrested cells continue to synthesize RNA, protein, and other macromolecules at normal growth rates. At the end of one generation time (2 h) of arrest, the mean volume of arrested cells is 1.6 times that of untreated cells (as expected for an asynchronously growing population with cells distributed approximately uniformly throughout the cell cycle). At hormone concentrations of less than 2 U/ml, many of the cells in the population recover from the transient G1 arrest during the 3-h incubation of the assay and reenter the cell cycle; at least in the case of α -factor, recovery appears to occur in part through proteolytic degradation of the hormone (5-7). The cell volume increase is a

function of the length of G1 arrest and thus reflects the length of time that some cells in the population can grow in size while arrested in G1 before the population as a whole recovers. Since asynchronous, exponentially growing cells are used, not all cells in the population (e.g., those just entering S phase) will be arrested at hormone concentrations of less than approximately 0.75 U/ml; these cells probably do not undergo a hormone-induced volume increase at all before recovery occurs. By using the Coulter Channelyzer, we can reproducibly detect volume increases at hormone concentrations as low as 0.05 U/ml. This assay is therefore far more sensitive than either the morphogenetic assay or the inhibition of cell division. Although the agglutination assay of Hartwell (17) is also quite sensitive, it is less convenient for large numbers of samples. The sensitivity of our assay would probably be further extended by using, as assay cells, *sst2* mutants that are supersensitive to the hormones (6).

Our initial difficulties with variability in *a*-factor assays apparently resulted from incomplete solubilization of the hormone during serial dilutions from the concentrated stock. When BSA was included in all dilutions and the assay mixture, with sonication at each dilution, the variability was greatly reduced. It is possible that BSA acts as a carrier protein for *a*-factor. Cell cultures may contain a specific or nonspecific carrier which permits the diffusion of *a*-factor through aqueous culture medium. The existence of such a carrier would explain the lower variability observed when unfractionated culture filtrates, rather than more purified preparations, are assayed for *a*-factor activity (Strazdis, thesis). However, we suspect that our assays may still underestimate the *a*-factor concentration, since the inclusion of octyl- β -D-glucopyranose in the assays (with or without BSA) usually resulted in more activity. However, it also introduced substantial variability between duplicates and a lack of reproducibility from experiment to experiment. Since none of the other detergents or alcohols tested enhanced *a*-factor activity, octyl- β -D-glucopyranose may affect the α assay cells instead of solubilizing *a*-factor.

The hydrophobicity of *a*-factor has also apparently hindered its reproducible recovery by purification methods that use ion-exchange chromatography, in which yields vary more than 10-fold (1, 3; (Strazdis, thesis)). By using Amberlite XAD-2 beads to adsorb *a*-factor from cell-free filtrates of a cultures, we obtained quite reproducible recoveries (800 U/ml) in different experiments (Table 3). When the beads were added to the culture at the beginning of growth, the yield of *a*-factor was significantly increased. In addi-

tion, adsorption of the hormone during growth shortened the time of preparation by 24 h and reduced the exposure of the hormone to aeration. This decreased aeration may be especially important during α -factor preparation because it has been found that the oxidized forms of α -factor, which contain methionine sulfoxide, exhibit significantly lower biological activity (26). The increased yields of both hormones could represent a concentration dependence in their synthesis (e.g., negative feedback regulation) or secretion or both or, alternatively, a reduction in degradation or oxidation. Betz and Duntze (1) have obtained three bands exhibiting *a*-factor activity on thin-layer plates. Two bands contained much less activity than the third, and the authors suggested that these two might represent degradation products of *a*-factor similar to those of α -factor in α culture filtrates (26).

The degree of purification obtained by adsorption to and elution from Amberlite XAD-2 with organic solvents is equivalent for both hormones, and they can both be purified further by chromatography on Sephadex LH-20, as described previously (1, 13). Comparable amounts of the hormone (5,100 U/liter of *a*-factor and 7,500 U/liter of α -factor) were recovered when the beads were added to the cultures at the beginning of growth. These results indicate that *a* cells are capable of secreting more *a*-factor than previously thought (3, 30), although the yield of *a*-factor adsorbed from cell-free filtrates suggests that its concentration in cell cultures may still be considerably lower than that of α -factor (800 versus 4,200 U/liter). The biological effects of the hormones on sensitive cells are quite similar, although we have observed one qualitative difference. By using the isolation and assay methods described in this paper, we have demonstrated that significantly more *a*-factor activity can be isolated from mixed cultures of *a* and α cells than from pure *a* cultures (Strazdis, thesis; J. R. Strazdis and V. L. MacKay, manuscript in preparation). Therefore, there may be an interaction between *a* and α cells which regulates the concentration of active hormone and creates optimal conditions for mating.

In summary, we have developed new methods for the isolation and assay of *a*-factor, a hydrophobic peptide mating hormone, as well as the less hydrophobic α -factor. The assay (0.375 ml) can be used for hormone concentrations between 0.05 and 2 U/ml and requires only small amounts of hormone (≥ 0.16 ng per 0.375-ml assay, as calculated from reference 1). It is convenient for large numbers of samples (>100) and is quite reproducible (approximately 16% variation) for a bioassay system, both within and between experiments. Improved isolation of *a*-factor was achieved by adsorption to and elution

from Amberlite XAD-2 beads, leading to yields much higher (up to 40-fold) and more reproducible (standard deviation of 12%) than those reported previously.

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