Expression and Characterization of the Flocculin Flo11/Muc1, a Yeast Mannoprotein with Homotypic Properties of Adhesion

Li Li Ph.D.
Molloy College, lli@molloy.edu
Lois M. Douglas
Yang Yang
A M. Dranginis

Follow this and additional works at: https://digitalcommons.molloy.edu/bces_fac
Part of the Biology Commons, and the Cell Biology Commons
DigitalCommons@Molloy Feedback

Recommended Citation
https://digitalcommons.molloy.edu/bces_fac/30

This Peer-Reviewed Article is brought to you for free and open access by the Biology, Chemistry, and Environmental Science at DigitalCommons@Molloy. It has been accepted for inclusion in Faculty Works: Biology, Chemistry, and Environmental Studies by an authorized administrator of DigitalCommons@Molloy. For more information, please contact tochtera@molloy.edu,thasin@molloy.edu.
Expression and Characterization of the Flocculin Flo11/Muc1, a Saccharomyces cerevisiae Mannoprotein with Homotypic Properties of Adhesion

Lois M. Douglas,‡ Li Li,‡§ Yang Yang, and A. M. Dranginis*

Department of Biological Sciences, St. John’s University, 8000 Utopia Parkway, Queens, New York 11439

Received 5 September 2006/Accepted 22 August 2007

The Flo11/Muc1 flocculin has diverse phenotypic effects. Saccharomyces cerevisiae cells of strain background 1278b require Flo11p to form pseudohyphae, invade agar, adhere to plastic, and develop biofilms, but they do not flocculate. We show that S. cerevisiae var. diastaticus strains, on the other hand, exhibit Flo11-dependent flocculation and biofilm formation but do not invade agar or form pseudohyphae. In order to study the nature of the Flo11p proteins produced by these two types of strains, we examined secreted Flo11p, encoded by a plasmid-borne gene, in which the glycosylphosphatidylinositol anchor sequences had been replaced by a histidine tag. A protein of approximately 196 kDa was secreted from both strains, which upon purification and concentration, aggregated into a form with a very high molecular mass. When secreted Flo11p was covalently attached to microscopic beads, it conferred the ability to specifically bind to S. cerevisiae var. diastaticus cells, which flocculate, but not to 1278b cells, which do not flocculate. This was true for the 196-kDa form as well as the high-molecular-weight form of Flo11p, regardless of the strain source. The coated beads bound to S. cerevisiae var. diastaticus cells expressing FLO11 and failed to bind to cells with a deletion of FLO11, demonstrating a homotypic adhesive mechanism. Flo11p was shown to be a mannoprotein. Bead-to-cell adhesion was inhibited by mannose, which also inhibits Flo11-dependent flocculation in vivo, further suggesting that this in vitro system is a useful model for the study of fungal adhesion.

The fungal adhesins are a family of cell surface proteins that mediate adherence to environmental substrates or to other cells (7, 45). Adhesins are critically important in the initial steps of fungal pathogenicity, when fungal cells must adhere to host tissue. For the common human pathogens Candida albicans and Candida glabrata, the involvement of multiple adhesins in the adherence of fungal cells to host tissue has been demonstrated (4, 5, 18, 26, 43).

Among the adhesins is the flocculin family of Saccharomyces cerevisiae cell wall proteins that mediate flocculation, which is assexual calcium-dependent cell-cell aggregation. The most recently described member of the yeast flocculin gene family, FLO11/MUC1 (24, 30), is the only flocculin expressed in the 1278b strain of S. cerevisiae (17), and it exhibits a wide variety of phenotypes. Some of these phenotypes are strain specific. Yeast cells of strain background 1278b have been shown to require FLO11 for invasive growth (23, 30), the development of pseudohyphae (24, 29), and the formation of biofilms on plastic (36), but they do not flocculate. On the other hand, the variant strain S. cerevisiae var. diastaticus, which is highly flocculent, has been shown to require FLO11 for flocculation (30).

FLO11 is also required in 1278b strains for the formation of mats with hub and spoke structures on semisolid agar (36). The common laboratory strain background S288C does not express FLO11 due to a nonsense mutation in the transcriptional activator FLO8 (28). In some industrial strains, FLO11 mediates formation of the specialized biofilms called flors that are necessary for the production of sherry wine (19, 48). The common feature of all these phenotypes is adhesion. Commensurate with the many different pathways that regulate its expression, FLO11 has been shown to have a promoter that is among the largest described for yeast, at over 3 kb (38). Much more is known about gene regulation of FLO11 (for reviews, see references 11, 25, and 32) than about the structure and function of the protein.

We further investigated the FLO11-dependent phenotypes of S. cerevisiae var. diastaticus and found that it also differs from 1278b in that the haploids do not invade agar and the diploids do not form pseudohyphae. In order to investigate these strain differences in the phenotypes of FLO11 we expressed and purified the Flo11 proteins from S. cerevisiae var. diastaticus and from 1278b and examined their properties. An in vitro system was created for studying the adhesive characteristics of the expressed Flo11 by attaching the protein to microscopic beads and testing the adhesive properties of the beads.

Microscopic beads that can be coated covalently with proteins or ligands have been used to simplify several complex biological processes. For example, Gaur and Klotz used microscopic magnetic beads coated with extracellular matrix proteins to isolate a C. albicans adhesion gene, ALS5, by expression cloning in S. cerevisiae (13). Further work using such beads
resulted in characterization of the adhesion properties of Ala1p and Asl1p (12, 14, 15, 21, 22, 35). In this study, we have used this approach to study the in vitro properties of purified Flo11 proteins from two different strains.

MATERIALS AND METHODS

Flo11 phenotypic assays. Flocculation was assayed by culturing cells to mid-log phase in yeast extract-peptone-dextrose medium (1), diluting them to equal cell densities, swirling them vigorously with a Vortex mixer, and photographing them. Flocculation was assayed by culturing cells to mid-log phase in yeast extract-peptone-dextrose medium (1), diluting them to equal cell densities, swirling them vigorously with a Vortex mixer, and photographing them immediately and at specified time intervals. Invasion of agar (37) and formation of biofilms on plastic (36) by haploid strains were assayed as described previously. Diploid strains were tested for pseudoporphys development on nitrogen starvation medium as described previously (16).

Construction of pFLO11-GPI. pFLO11-GPI was derived from Yeplac181-PGK1p-MUC1 (abbreviated here as pFLO11), which was generously provided by the laboratory of Isak S. Pretorius (8). Yeplac181-PGK1p-MUC1 is a 2 μm LEU2 mutant yeast-Enhancer coi shuttle vector containing FLO11 regulated by the constitutive PGK1 promoter (8). The ultimate source of the FLO11 gene in this plasmid is codon clone ATCC 70895 (American Type Culture Collection). The FLO11 sequence is identical to that found by the yeast genome sequencing project in strain background S288C (24).

Growth of yeast strains. Yeast strains were grown in SC media (1) or in SC media supplemented with amino acid supplements suggested by the manufacturer for dihydrophosphatidylglycerol synthesis. Yeast cells were harvested in early logarithmic growth phase (2× SC). Cultures were centrifuged at 3,000×g for 5 min at 4°C and were resuspended in 100 μl of fresh medium at an optical density of 1.0 at 600 nm. Cells were cultured for 200 h in the presence of 100 μl of blocking reagent (Roche Diagnostics, Mannheim, Germany) and 10 ml buffer 2 (0.1 M Tris-HCl–0.05 M NaCl, pH 7.5), and washed one time for 10 min in TBS (0.05 M Tris-HCl-0.15 M NaCl, pH 7.5). Ten microliters of digoxigenin-labeled GNA (Roche Diagnostics, Mannheim, Germany) was added to 10 μl buffer 1, and the blot was incubated in the solution for 1 h at room temperature. Yeast cells were resuspended in 100 μl of blocking reagent (Roche Diagnostics, Mannheim, Germany), washed two times for 10 min each in 50 ml buffer 1 (TBS-1 mM MgCl2-1 mM MgCl2, pH 7.5), and washed one time for 10 min in TBS (0.05 M Tris-HCl-0.15 M NaCl, pH 7.5). The blot was incubated in a substrate solution containing 200 μl of 4-nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolylphosphate (Roche Diagnostics, Mannheim, Germany) and 10 μl buffer 2 (0.1 M Tris-HCl-0.05 M MgCl2-0.1 M NaCl, pH 9.5) until color development was complete.

Preparation of Flo11-coated beads. Dynal M-450 tosyl-activated beads (Dynal Biotech, Inc., Lake Success, NY) are polystyrene beads that are approximately the size of yeast cells (4.5 μm in diameter). The surface of each bead is activated with p-toluenesulfonyl chloride to facilitate the covalent binding of proteins through their amino or sulfhydryl groups. Beads at a concentration of 4 × 109/ml were pipetted in 25-μl aliquots into 0.5-ml tubes. Beads were collected using a strong magnet (Magnetight separation stand; Novagen, Inc., Madison, WI) for 1 to 2 min, and supernatants were discarded. The beads were gently resuspended in 100 μl of buffer A (0.1 M sodium phosphate, pH 7.4) for 2 min

TABLE 1. Yeast strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Yeast strain or plasmid</th>
<th>Strain background</th>
<th>Genotype or description</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>L5487</td>
<td>Σ1278b</td>
<td>MATa leu2::hisG ura3::52</td>
<td>37</td>
</tr>
<tr>
<td>L5487 flo11Δ</td>
<td>Σ1278b</td>
<td>MATa leu2::hisG ura3::52 flo11::URA3</td>
<td>29</td>
</tr>
<tr>
<td>L5489</td>
<td>Σ1278b</td>
<td>MATa/s1278::hisG ura3::52 flo11::URA3</td>
<td>C. Styles and G. Fink</td>
</tr>
<tr>
<td>Y1Y345</td>
<td>S. cerevisiae var. diastatiscus</td>
<td>MATa ura3 leu2-3,112 his4 sta1</td>
<td>47</td>
</tr>
<tr>
<td>Y1Y345 flo-1</td>
<td>S. cerevisiae var. diastatiscus</td>
<td>MATa ura3 leu2-3,112 his4 sta1 flo11::URA3</td>
<td>30</td>
</tr>
<tr>
<td>YC886</td>
<td>S. cerevisiae var. diastatiscus</td>
<td>MATa leu2-3,112 ura3::Δ227 ary1 ST1</td>
<td>6</td>
</tr>
<tr>
<td>M1800D × Y1Y319</td>
<td>S. cerevisiae var. diastatiscus</td>
<td>MATa/s1278::hisG ura3::52 leu2Δ1</td>
<td>46</td>
</tr>
<tr>
<td>FY6S</td>
<td>S288C</td>
<td>MATa his3Δ200 ura3::52 leu2Δ1</td>
<td>6</td>
</tr>
</tbody>
</table>

Plasmids

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Markers</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pFLO11</td>
<td></td>
<td>This work; derived from Yeplac181-PGK1p-MUC1 (8)</td>
</tr>
<tr>
<td>pFLO11-GPIΔ</td>
<td></td>
<td>This work; derived from Yeplac181-PGK1p-MUC1-GPIΔ (8)</td>
</tr>
</tbody>
</table>
and collected on the magnetic stand for 1 minute. Buffer was pipetted off and discarded. Five micrograms of Flo1lp was added to each tube, i.e., to 1 × 10^7 washed beads, along with buffer A to maintain the initial total volume of 25 μl. Each tube was vortexed for 2 min and incubated at 30°C for 16 to 24 h with slow, tilt rotation. Beads were also incubated without added Flo1lp as a control. Upon completion of incubation, beads were collected for 2 to 3 min in the magnetic stand, and supernatants were removed. Beads were washed twice for 5 min each with buffer A to maintain the initial total volume of 25 μl.

**RESULTS**

**Yeasts from strain backgrounds Σ1278b and S. cerevisiae var. diastaticus differ in expression of FLO11-dependent phenotypes.** It was previously shown that Σ1278b strains express FLO11 (29), as do strains of S. cerevisiae var. diastaticus (30). However, the phenotypic expression of FLO11 differs in these two strains. S. cerevisiae var. diastaticus strains flocculate very strongly in a FLO11-dependent way, as they begin to settle out of solution immediately after being mixed (Fig. 1A). Σ1278b strains are virtually nonflocculent in comparison, remaining entirely suspended even 2 h after being mixed (Fig. 1A). Σ1278b diploid strains, on the other hand, invade agar and form pseudohyphae (16, 17), while S. cerevisiae var. diastaticus strains do not (Fig. 1B and C). Both S. cerevisiae var. diastaticus strains and Σ1278b (36) form biofilms on polystyrene microtiter wells (Fig. 1D). To explore the basis for these
strain-specific differences in phenotypic expression, we purified the Flo11 protein from each strain and tested its properties.

A GPI anchor is required for cell surface localization of Flo11. Many fungal adhesins localize to the cell surface through either a cell membrane-anchored or a cell wall-anchored GPI moiety (31). Flo11p has been localized to the yeast cell wall (17, 30). We replaced the GPI anchor of a histidine tag to facilitate secretion of the protein into the medium, from which it could then be purified using nickel columns. When the GPI anchor of FLO11 was replaced by a six-histidine tag, yeast cells of strain background \( \Sigma 1278b \) transformed with GPI anchorless FLO11 (pFLO11-GPI\( \Delta \)) secreted Flo11p (Fig. 2A, lane 1), whereas isogenic yeast strains transformed with wild-type FLO11 (pFLO11) did not secrete Flo11p (Fig. 2A, lane 2). Using the same plasmids in \( S.\ cerevisiae \) var. \( \textit{diastaticus} \) also resulted in secretion of the protein (Fig. 2C). This protein was by far the most abundant protein in the concentrated medium, as shown by Coomassie blue staining of the gels in Fig. 2. Therefore, the GPI anchor is critically required for cell surface localization of Flo11p, and its removal results in the accumulation of significant amounts of the protein in the medium.

Secreted Flo11p forms aggregates. The culture supernatant of \( \Sigma 1278b \) strain L5487 flo11\( \Delta \) transformed with pFLO11-GPI\( \Delta \) contained a protein of approximately 196 kDa (Fig. 2A, lane 1). The identity of the protein as Flo11p was verified by immunodetection with anti-His antibody. However, a much higher-molecular-mass form of Flo11p (\( \geq 220,000 \) Da) was detected after the same supernatant was purified on a nickel column (Fig. 2B). Fractions collected from the nickel column are shown in Fig. 2B. The higher-molecular-weight form remained in the stacking gel. Total protein quantification showed that purification over Ni-nitrilotriacetic acid resin concentrated Flo11p approximately three times (up to 35 \( \mu g/ml \) in peak fractions) (data not shown). The culture supernatant of \( S.\ cerevisiae \) var. \( \textit{diastaticus} \) strain YIY345 transformed with pFLO11-GPI\( \Delta \) most often exhibited the high-molecular-weight form of Flo11p, even before column purification (Fig. 2C, lane 3), although it was also observed in the 196-kDa form (data not shown). Once formed, this high-molecular-weight form of the protein could not be disaggregated by any of the methods tried, which included varying the temperature of incubation of the protein samples, the addition of EDTA at up to 500 mM, and the addition of 50% formamide. Formation of the high-molecular-weight form of Flo11p was also not dependent on the stage of culture growth or the cell density (data not shown). Cell wall-adhesive glycoproteins are known to form aggregates such as this (3, 39, 42).

Flo11p is a mannoprotein. One common characteristic of fungal adhesins is that they contain a large number of residues that are susceptible to N- or O-linked glycosylation (31). Flo11p contains a central domain in which about 50% of the residues are serine and threonine, which are potential O-linked glycosylation sites. Since the predicted molecular mass of Flo11p derived from its amino acid sequence is 137,000 Da, the larger mass observed on the stained gels and immunoblots suggests that the FLO11 protein product is modified. To test for mannose modification, purified Flo11p samples from YeAD5 (\( S.\ cerevisiae \) var. \( \textit{diastaticus} \) background) and L5487 flo11\( \Delta \) (\( \Sigma 1278b \) background) transformed with pFLO11-GPI\( \Delta \) were blotted onto PVDF membranes and treated with digoxigenin-labeled GNA, a plant lectin that specifically binds to \( (1-3)-, (1-6)-, \) and \( (1-2)-\) linked terminal mannose residues. Figure 3 shows that Flo11p proteins from both yeast strains are mannoproteins possessing terminal mannose residues (Fig. 3, lanes 1 and 2). The positive control for this assay was carboxypeptidase Y (Fig. 3, lane 5), a known manno-

![FIG. 2. SDS-polyacrylamide gel electrophoresis of Flo11 proteins secreted from yeast cells of two strain backgrounds. In each case, the Coomassie-stained polyacrylamide discontinuous gel (3.9% stacking gel and 5% resolving gel) is shown on the left, while the corresponding Western blot using anti-six-His antibody is shown on the right. (A) Lane 1, culture supernatant of strain L5487 flo11\( \Delta \) (\( \Sigma 1278b \) background) transformed with pFLO11-GPI\( \Delta \); lane 2, culture supernatant of L5487 flo11\( \Delta \) transformed with pFLO11 (GPI anchor sequences included); lane 3, protein molecular size marker. (B) Purified secreted Flo11p from the \( \Sigma 1278b \) strain aggregated in a form that remained in the stacking gel. Secreted Flo11p from strain L5487 flo11\( \Delta \) transformed with pFLO11-GPI\( \Delta \) was purified on a nickel column. Lane 1, protein size marker; lane 2, fraction 7; lane 3, fraction 8; lane 4, fractions 9 and 10; lane 5, fractions 13 and 14. (C) Secreted Flo11p from \( S.\ cerevisiae \) var. \( \textit{diastaticus} \) strain YIY345 flo1-1 transformed with pFLO11-GPI\( \Delta \) produced a large form that remained in the stacking gel. Lane 1, protein size marker; lane 2, culture supernatant of YIY345 transformed with pFLO11; lane 3, culture supernatant of strain YIY345 transformed with pFLO11-GPI\( \Delta \).](https://ec.asm.org/content/6/21/2217.full.pdf)
tein. The glycoproteins fetuin and transferrin, which do not contain mannose, served as negative controls.

Beads coated with Flo11p derived from either \( \Sigma 1278b \) or \( S. \) cerevisiae var. diastaticus bind \( S. \) cerevisiae var. diastaticus cells. Yeast strain \( \Sigma 1278b \) and \( S. \) cerevisiae var. diastaticus both express the \( FLO11 \) gene (unlike the standard laboratory strain \( S288C \)), but only \( S. \) cerevisiae var. diastaticus exhibits Flo11-dependent flocculation. One hypothesis is that the Flo11 proteins produced in these two strains are modified or localized differently, enabling only one form to mediate flocculation. Another possibility is that additional factors in the yeast cell wall are responsible for the different activities of Flo11p in these strains.

In order to examine these hypotheses, an in vitro model system was created for the study of Flo11-dependent adhesion by coating plastic beads with Flo11p secreted from the two strains of yeast. The Flo11 protein secreted into the medium by \( pFLO11-GPI \)-bearing cells was covalently bound to polystyrene beads of approximately the size of a yeast cell. Dynal M450 tosyl-activated magnetic beads (4.5 \( \mu \)m in diameter) (Dynal Biotech, Inc., Lake Success, NY) are derivatized with \( p \)-toluenesulfonyl chloride to facilitate the covalent binding of proteins to the surfaces of the beads. The Flo11 protein produced by both \( \Sigma 1278b \) and \( S. \) cerevisiae var. diastaticus plasmid-bearing cells was used to coat these beads. Since the secreted Flo11p was encoded by the plasmid, it would have the same primary sequence in both strains, which is identical to the sequence from the \( S288C \) strain background, as determined by the yeast genome sequencing project (24). The coated beads were then tested for the ability to adhere to cells of various types.

Uncoated beads did not bind to cells of either strain (see Fig. 5A). However, when they were coated with Flo11p from either yeast strain, the beads adhered to \( S. \) cerevisiae var. diastaticus cells (Fig. 4, top row; and 5A). Therefore, purified Flo11p is sufficient to bind to the cell wall of \( S. \) cerevisiae var. diastaticus. Flo11p produced by the nonflocculent strain \( \Sigma 1278b \) was fully capable of mediating bead-to-cell adhesion. Furthermore, the same result was achieved whether the 196-kDa form of Flo11 (from \( \Sigma 1278b \)) or the large form (>220 kDa; from purified

FIG. 4. Bead adhesion assay. Flo11p-coated beads bound specifically to \( S. \) cerevisiae var. diastaticus cells in a \( FLO11 \)-dependent manner. Dynal beads coated with purified secreted Flo11p from either the \( S. \) cerevisiae var. diastaticus or \( \Sigma 1278b \) strain background were added to different strains of yeast cells, mixed, and photographed. All photos were taken with a 40X objective in the differential interference contrast setting. Beads (approximately 4.5 \( \mu \)m) are visible as dark particles. Cells used were from the following strains: \( S. \) diastaticus w.t., strain YIY345; \( S. \) diastaticus flo11Δ, strain YIY345 flo-1; \( \Sigma 1278b \) w.t., strain LS487; \( S288C \) w.t., strain FY86. A quantitative analysis of this assay is shown in Fig. 5A.

FIG. 5. (A) Quantitation of bead adhesion assay. Each column was based on the mean ± standard error for three independent experiments, with at least 200 beads counted in each. Uncoated beads (white bars) showed no specific binding to cells; beads coated with purified Flo11p secreted from either \( S. \) cerevisiae var. diastaticus strain YIY345 (gray bars) or \( \Sigma 1278b \) strain LS487 flo11Δ (black bars) showed specific binding to \( S. \) cerevisiae var. diastaticus wild-type cells. (B) Mannose inhibits adhesion of coated beads to yeast cells, just as it inhibits flocculation of these cells. Beads coated with Flo11p derived from \( S. \) cerevisiae var. diastaticus strain YIY345 were added to cells of strain YIY345, with and without mannose treatment, and the numbers of beads bound and not bound to cells were counted for each case.

In order to test these hypotheses, an in vitro model system was created for the study of Flo11-dependent adhesion by coating plastic beads with Flo11p secreted from the two
Flo11, a Mannoprotein, Exhibits Homotypic Adhesion

Vol. 6, 2007

The adhesion assays were quantitated by counting adherent beads and nonadherent beads in a mixture of cells and beads, using a microscope. The average of three independent assays for each mixture is shown in Fig. 5A. Flo11p from the two strains, i.e., Σ1278b and S. cerevisiae var. diastaticus, was shown to bind similarly to cells. Among the in vivo properties of Flo11-expressing cells is the ability to adhere to agar (24, 29) and to polystyrene (36). These Flo11-coated beads, however, were not observed to bind to agar or plastic (data not shown). It may be that the proper experimental conditions for such binding have not been found, that the specific gravity of the magnetic beads is too high to permit adhesion, or that other factors are involved in binding to these substrates.

Mannose inhibits Flo11-dependent adhesion. Since mannose inhibits Flo11-dependent flocculation in vivo (2), an in vitro adhesion assay was performed to directly assess the ability of D-mannose to affect the binding of Flo11-coated beads to yeast cells. When beads coated with Flo11p derived from S. cerevisiae var. diastaticus strain YeAD5 were combined with S. cerevisiae var. diastaticus Y1Y345 cells and then incubated in 1 M D-mannose, no binding of beads to cells was detected (Fig. 5B). Glucose at the same concentration did not inhibit binding (data not shown). Flo11p is a member of the class of Flo1p-type flocculins, whose defining characteristic is inhibition by mannose but not by glucose, maltose, or sucrose (2). Therefore, this in vitro system utilizing coated beads to test Flo11p adhesion to cells reflects the in vivo properties of mannose inhibition. This finding provides further evidence that mannose is a component to which Flo11p binds on the cell and that Flo11p functions in a lectin-like manner in S. cerevisiae var. diastaticus.

DISCUSSION

This study directly demonstrates that Flo11p is an adhesion molecule. Yeast cells of three different strain backgrounds were used in these studies. S288C has been used as a standard laboratory strain of yeast for many years; it does not express FLO11 and consequently does not form pseudohyphae or exhibit other FLO11-dependent properties (28, 38). The first yeast strain shown to form pseudohyphae and to invade a substrate was Σ1278b (16), which exhibits constitutive suppression of the stress response due to high cyclic AMP levels (40). Cells with the Σ1278b background exhibit many FLO11-dependent characteristics besides pseudohyphae, including agar invasion (29, 37), biofilm formation, and adhesion to plastic (36), but they do not flocculate. Saccharomyces cerevisiae var. diastaticus, on the other hand, flocculates very strongly, but when FLO11 is deleted flocculation is abolished (30). However, this work shows that in spite of its expression of Flo11p, S. cerevisiae var. diastaticus does not invade agar or form pseudohyphae. These two strains thus represent a naturally occurring experiment to determine the factors that govern Flo11-dependent adhesion. In order to investigate these factors, Flo11 proteins were purified from these two strains and their properties were tested.

When the GPI anchor sequences were removed from Flo11p, the protein accumulated in the extracellular medium. Two forms of Flo11p were observed, with one form of 196 kDa and another, very large form, which did not enter the separating gel. Purification of secreted Flo11p on nickel columns always resulted in conversion of the 196-kDa form to the larger form. We suggest that the large form represents an aggregate of Flo11 protein. Such aggregates have frequently been seen in SDS gels containing membrane and cell wall proteins (3, 9, 33, 39, 42). At least three groups have purified mannose-specific lectins from yeast surfaces, and similar SDS-resistant aggregation properties were displayed by all of them (3, 39, 42). Bony et al. (3) extracted Flo1p from cell walls by using hot SDS–β-mercaptoethanol and observed that the protein migrated in SDS gels as a very high-molecular-mass protein in a highly heterogeneous fashion. The fastest-migrating forms of Flo1p exhibited molecular masses of about 200 kDa, while the largest forms remained at the top and did not enter the resolving gel (3), just as we found for Flo11p. When the GPI anchor sequences of Flo1p were removed, the protein was secreted into the medium in a form that produced a fuzzy, heterogeneous, high-molecular-mass band in gels (3). Treatment with endo-β-N-acetylglucosaminidase H resulted in more protein entering the gel, suggesting that Flo1p is N glycosylated. However, the enzyme-treated Flo1p remained larger than predicted from the amino acid sequence, suggesting O glycosylation as well (3).

Consistent with the model of flocculins as glycoproteins, a homolog of Flo1p has been demonstrated to have a sugar content of 63% (42). This protein also exhibited properties of aggregation: gel filtration studies revealed an active aggregate with an apparent molecular mass of >700 kDa. The present study establishes Flo1lp as a mannoprotein.

Flo1lp functioned as an adhesin in vitro when it was attached to beads. The coated beads bound only to cells of the strain background that exhibits Flo11-dependent flocculation, namely, S. cerevisiae var. diastaticus, not to Σ1278b strains, which do not flocculate. This binding was not due to nonspecific trapping of the beads in the large flocs of S. cerevisiae var. diastaticus, since uncoated beads did not bind. Both strains produced Flo1lp that is mannosylated, and both produced...
Flo1p that adheres to *S. cerevisiae* var. *diastaticus* cells equally well. In vitro model systems such as this one should prove to be very useful for further understanding fungal adhesion.

The work presented here is the first to identify an adhesive target of Flo1p. Since the outer layer of the yeast cell wall consists largely of mannoproteins (20, 27), it seemed likely that Flo1p would bind to the side chains of the cell wall mannoproteins. It has been thought that the specificity of fungal lectins may be quite broad (41). Somewhat surprisingly, we found that an essential component (and perhaps the only component) of the adhesive target of Flo1p is, in fact, other Flo1p molecules. Flo1p receptors on the cell wall are required for adhesion of Flo1-coated beads. It remains to be seen whether they are sufficient. Strain-specific differences in these Flo1p receptors in the cell wall could possibly explain the differential adhesion of *S. cerevisiae* var. *diastaticus* versus Σ1278b cells to the coated beads. Such receptor differences could be quantitative or qualitative.

Homotypic adhesion has been demonstrated for the ALS adhesins of *Candida albicans* (21, 22). Yeast cell aggregation mediated by cloned ALS proteins has properties characteristic of amyloid protein aggregation (35), and alterations in these properties could, in theory, produce phenotypic variation. Another possible mechanism of fungal adhesion is suggested by the observation that in Als5p, the threonine-rich repeat domain of amyloid protein aggregation (35), and alterations in these properties similar to those of *Candida albicans* adhesin function. Immune. Immunol. 65:5289–5294.


