

## Degenerate Peptide Recognition by *Candida albicans* Adhesins Als5p and Als1p

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***Candida albicans* and *Saccharomyces cerevisiae* expressing the adhesins Als5p or Als1p adhere to immobilized peptides and proteins that possess appropriate sequences of amino acids in addition to a sterically accessible peptide backbone. In an attempt to further define the nature of these targets, we surveyed the ability of yeast cells to adhere to 90- $\mu$ m-diameter polyethylene glycol beads coated with a 7-mer peptide from a library of 19<sup>7</sup> unique peptide-beads. *C. albicans* bound to ca. 10% of beads from the library, whereas *S. cerevisiae* expressing Als5p or Als1p bound to ca. 0.1 to 1% of randomly selected peptide-beads. *S. cerevisiae* expressing Als1p had a distinctly different adherence phenotype than did cells expressing Als5p. The former adhered in groups or clumps of cells, whereas the latter adhered initially as single cells, an event which was followed by the build up of cell-cell aggregates. Beads with adherent cells were removed, and the peptide attached to the bead was determined by amino acid sequencing. All adhesive beads carried a three-amino-acid sequence motif ( $\tau\phi+$ ) that possessed a vast combinatorial potential. Adherence was sequence specific and was inhibited when soluble peptide identical to the immobilized peptide was added. The Als5p adhesin recognized some peptides that went unrecognized by Als1p. The sequence motif of adhesive peptides identified by this method is common in proteins and offers so many possible sequence combinations that target recognition by the Als proteins is clearly degenerate. A degenerate recognition system provides the fungi with the potential of adhering to a multitude of proteins and peptides, an advantage for any microorganism attempting to establish a commensal or pathogenic relationship with a host.**

The adherence of *Candida albicans* to human tissue is an important characteristic of this fungus for its life as both a commensal and an opportunistic pathogen (1, 17). Adherence may be mediated by a variety of proteins expressed on the surface of the microorganism, including up to eight members of the *Agglutinin-Like Sequence (ALS)* gene family (20). Two of the proteins encoded by these genes, Als5p and Als1p, can mediate adherence of the nonpathogenic yeast *Saccharomyces cerevisiae* to human cells and extracellular matrix components (4, 6). (In previous publications we referred to *ALS5* as *ALAI* or *ALAI/ALS5* [5, 6, 7].) Als5p mediates the initial adhesion of yeast cells to extracellular matrix protein-coated substrates and human buccal epithelial cells and also cell-cell aggregation, a feature that follows adherence of a single yeast cell to a fixed target (5). Fu et al. refer to the cell-cell association mediated by Als1p as flocculation (3). In a model of oral pharyngeal candidiasis *C. albicans* cells bearing knockouts of *ALSI* adhered less than wild-type cells to the mouse tongue, supporting the concept that an Als protein is important to the adherence process both in vivo and in vitro (10).

Given these findings, how does one go about determining the molecular mechanisms of adherence of *C. albicans*? One approach is to perform knockouts of selected genes, as has been done with *ALSI* (3, 10). Because of the large number of *ALS*

genes in *C. albicans* we have chosen a different approach, i.e., studying *C. albicans* alongside *S. cerevisiae* expressing different *Candida ALS* genes. The adhesive properties of *S. cerevisiae* cells expressing either Als5p or Als1p mimic the properties of *C. albicans* clinical isolates (5). The *C. albicans* adhesin proteins Als5p and Als1p are sufficient to mediate fungal cell adhesion to extracellular matrix, human cells, and cell-cell aggregation to form microcolonial structures (4–6). This finding has allowed us to delineate the behavior of these proteins when present as the sole active cell surface adhesin when expressed in *Saccharomyces* spp. Furthermore, this avoids the possibility of unintended and undetected changes introduced by knockout procedures. This same method of heterologous expression has been used to characterize an adhesin of *C. glabrata* (2).

The binding specificity of Als5p has been partially characterized. Adherence mediated by Als5p occurs within minutes, is strong enough to resist vortexing and is reversibly inhibited by denaturing agents such as formamide, high pH, or urea (5). Adherence with Als5p occurs to a number of amino acid sequences that are characterized, in part, by their steric configuration. For example, homopolymers of serine, threonine, or alanine (6-mers or larger) make ready targets for adherence when immobilized on bead surfaces (7). When these same peptides are formed into a closed loop with the same number of residues, the patches are not “recognized,” and adherence does not ensue (7). Adherence occurs with naturally occurring sequences as well, e.g., that of the serine/threonine-rich fibronectin peptide, FTTTST STPV (7). Thus, both sequence and conformation are important ligand recognition characteristics.

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Despite the similar amino acid sequence and adherence properties of Als5p and Als1p, there are differences in ligand recognition. *S. cerevisiae* expressing Als5p or Als1p adhered to magnetic beads coated with peptides in very different numbers (7; V. L. Chan, S. A. Klotz, N. K. Gaur, and P. B. Byrd-Williams, Abstr. Sixth Annu. Candida Candidiasis Meet., abstr. 121, p. 74, 2002). For example, *S. cerevisiae* expressing Als5p adhered in small numbers, whereas *C. albicans* and *S. cerevisiae* expressing Als1p adhered and aggregated in great numbers to beads bearing FTTTSTSTPV. Indeed, Als1p-expressing cells can flocculate in the absence of an added ligand, whereas cells bearing Als5p do not (3, 5). Furthermore, cells expressing Als5p or Als1p recognize and adhere to peptides that do not contain sequences rich in serine, threonine, or alanine. Such an adherent peptide was APRLRFYSL (7). These observations suggest that (i) Als5p and Als1p recognize and bind to many different amino acid sequences and (ii) different Als adhesins may recognize and bind to different amino acid sequences with different affinities.

Therefore, we studied the adherence of *C. albicans* and *S. cerevisiae* yeast cells expressing either Als protein to specific peptides identified from a random, polyethylene glycol (PEG)-bead based peptide library. The results show that the two adhesins recognize a broad array of target ligands. The ability of the yeast cells to adhere to a multitude of peptide ligands indicates that the "adherence recognition system" of these adhesins is degenerate, i.e., neither highly specific nor highly organized as to their protein or peptide targets. Such a recognition system provides the microorganism the ability to adhere to a large repertoire of targets and is entirely consistent with previous in vitro measurements of *C. albicans* adherence to proteins and peptides.

#### MATERIALS AND METHODS

**Bead-peptide library.** 7-mer peptides were synthesized onto PEG beads (~90- $\mu$ m diameter) according to the method of Lam et al. (16). The complete library consists of 19<sup>7</sup> unique beads and peptides (cysteine is not used in synthesis in order to avoid cross-linking of peptides). The library was washed thoroughly with Tris-EDTA (TE) buffer and stored at 4°C until use.

**Microorganisms.** *C. albicans* strain CA1 was grown in liquid yeast extract-peptone-dextrose. *S. cerevisiae* YPH499 or *S. cerevisiae* YPH499 expressing Als5p or Als1p were grown in liquid yeast extract-peptone-raffinose-galactose medium at 28°C prior to adherence assays as described previously (5, 7). The vectors of Als5p and Als1p are low-copy vectors, and both genes are expressed from GAL1 promoters. Cells were thoroughly washed with TE buffer by centrifugation and suspended at 10<sup>9</sup>/ml prior to use. We have used numerous biological buffers in this adherence assay; all are equivalent (6), and the adherence of the fungal cells is not affected by the addition of sugars or Tween 20 to the incubation mixture. Hence, the binding described in not significantly affected by hydrophobic or lectin interactions. However, yeast cell adherence to the beads is readily reversible by the addition of agents or conditions that break hydrogen bonds, e.g., formamide, high pH, and urea (5, 7). TE buffer is used in order to reduce the spontaneous cell-cell aggregation of *S. cerevisiae* expressing Als1p. *S. cerevisiae* YPH499 always served as a negative control.

**Assay.** Six-well culture plates were used for the adherence assay. A total of 20  $\mu$ l of beads (ca. 1,000 beads) and 1  $\mu$ l of yeast (~10<sup>6</sup> cells) was added to each well containing 1.5 ml of TE buffer. The culture plate was then agitated for 30 min on a rotary shaker at 150 rpm at room temperature. For the bovine serum albumin (BSA) experiments, 10<sup>7</sup> yeast cells were mixed with 100  $\mu$ g of BSA/ml either in native conformation or denatured by boiling for 10 min in TE buffer. Beads were observed by inverted microscopy for adherent fungi and removed with a plastic pipette.

Scoring of adherence was done with the following notations: -, no adherence; +, adherence of 10 to 25 yeast cells; ++, bead almost covered by 80 to 150 yeast cells; and +++, adherence with aggregation of yeast cells. Four different observers were tested, and each reported similar results in the scoring the assay.

**Identification of peptides.** Beads with adherent yeast cells were treated with 8 M guanidine-HCl for 30 min, washed thoroughly with phosphate-buffered saline, and submitted to the Laboratory for Protein Sequencing and Analyses, Department of Chemistry, University of Arizona, for sequence analysis of the attached peptide. Confirmation that the peptide sequences were responsible for adherence was obtained by specific synthesis of PEG beads bearing the sequence to be tested. Adherent sequences resulted in yeast cells adhering in large numbers to >95% of the beads, thus confirming the sequence as adherent.

(These findings were presented in part at the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, Calif., September 2002.)

#### RESULTS

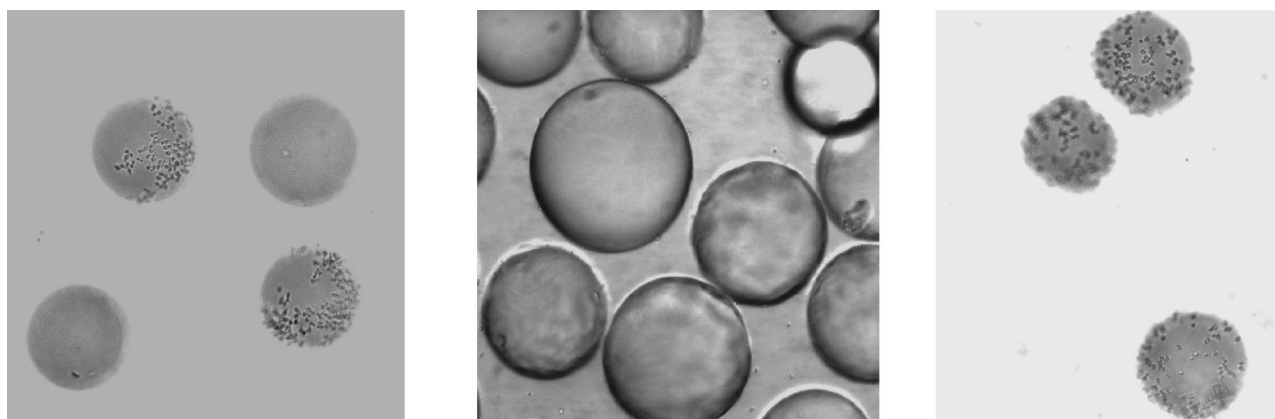
**Detecting multiple adherent peptide ligands.** The assay consisted of mixing *C. albicans* yeast cells with ~1,000 beads from the 7-mer bead-peptide library in 1.5 ml of TE buffer, all placed in the well of a six-well tissue culture tray. More than 150 wells were observed in this manner with the library beads and bead selection attempted. In each well with its random sample of 1,000 library beads, usually 5 to 100 beads were coated with various amounts of *C. albicans*. However, the vast majority of beads had no adherent *C. albicans* whatsoever (Fig. 1, left panel). *S. cerevisiae* expressing Als5p or Als1p adhered to one to three beads per 1,000 beads from the 7-mer library. *S. cerevisiae* not expressing an Als protein did not adhere to any peptide-beads (Fig. 1, middle panel). In addition, *S. cerevisiae* expressing the vector without *Candida* DNA did not adhere to protein or peptide coated beads (6); and no yeast cell (*Candida* nor *Saccharomyces*) adhered to beads lacking a peptide. As another negative control we sequenced the peptide on a bead that had not bound yeast cells. A population of beads bearing the sequence EHAHTPR did not bind *C. albicans* or *S. cerevisiae* expressing either adhesin. If a confirmed sequence was used, >95% of the beads were covered by yeast cells (for example, beads bearing PIANMRK, Fig. 1, right panel [boldfacing is defined below]).

Since a number of library beads were binding yeast cells to their surface, it was apparent that this bead-fungus interaction had fairly broad specificity. We randomly chose five beads that bound *C. albicans* from a screening of 50,000 beads, and the peptides on these beads were sequenced and confirmed (see Materials and Methods) as adhesive ligands (Table 1). Similar screening and random selection of beads with adherent *S. cerevisiae* expressing Als5p or Als1p was performed and identified six and three peptide ligands, respectively (Table 1).

All peptides identified as ligands in this search had a sequence motif " $\tau\phi+$ ," with " $\tau$ " representing a residue with high turn propensity (A, D, G, K, N, P, or S), " $\phi$ " representing a bulky hydrophobic or aromatic residue (F, H, I, L, M, T, V, W, and Y), and "+" representing R or K. In Table 1 and throughout the remainder of the text this sequence motif is denoted in boldface type.

Yeast cells were reversibly bound to the beads. This was demonstrated by treatment of beads decorated with yeast cells with 50% formamide in water. This treatment dissociates the adherent yeast cells. Upon removal of the formamide and replacing it with buffer, the yeast cells rapidly reattached to the beads (data not shown).

**Specificity of ligand sequences.** We have reported previously on the use of tosyl- and carboxylate-activated magnetic beads in peptide-fungal adherence assays (7). The tosyl-activated beads are 5  $\mu$ m in diameter, the carboxylate-activated beads



*Candida albicans*  
peptide: 7-mer library

*Saccharomyces cerevisiae*  
peptide: DKGWRAP

*Saccharomyces cerevisiae*  
expressing Als5p  
peptide: PIANMRK

FIG. 1. Left panel: *Candida albicans* yeast cells adhering to the surface of 2 PEG beads from the 7-mer library. Two of the library beads are not recognized by *C. albicans*. Middle panel: *Saccharomyces cerevisiae* mixed with beads coated with DKGWRAP. The yeast is expressing no *Candida* DNA. None of the beads have yeast cells adherent to them. Right panel: *S. cerevisiae* expressing Als5p mixed with beads bearing the peptide, PIANMRK. All beads are coated with yeast cells, some of which are aggregating.

are 0.7 μm in diameter, and in each case the peptides are covalently bound at the N terminus of the peptides. The adherence of yeast cells to the tosyl- and carboxylate-activated beads was inhibited with a soluble peptide of the same sequence as the bead-bound peptide. The PEG beads used in this assay are ~90 μm in diameter, and the peptides are covalently bound at the C terminus. Therefore, **KLRIPSV**, a PEG-bead-bound peptide recognized by *C. albicans*, was coupled to the carboxylate-modified magnetic beads at the N terminus and tested for adherence. *C. albicans* recognized and adhered to the peptide attached in this configuration as well (data not shown). Thus, neither the type of bead nor the mode of coupling qualitatively affected recognition of an adhesive peptide by *C. albicans*.

Because the adhesive peptides had similar amino acid compositions (see Discussion), we determined whether adhesion was mediated by composition alone. Accordingly, we synthesized a set of beads bearing the sequence SKMAYTL, a scrambled version of the sequence **AYKSLMT** that was determined to be adhesive for all three adhesin-expressing cell types (Fig. 2, left panel). The scrambled sequence was poorly adhesive for either adhesin when expressed in *S. cerevisiae* (Fig. 2, middle

panel) and showed slightly reduced binding for *C. albicans* (Table 2). Sequence specificity was also demonstrated by peptide competition studies. The soluble heptamer VTHTHRR (5.5 × 10<sup>-4</sup> M) effectively inhibited adherence of Als5p-bearing *S. cerevisiae* cells to homologous beads (Table 3). The high concentration of the peptide required for specific blocking in this instance demonstrates the weak interaction of the adhesin with the target peptide when in the nonimmobilized state. *C. albicans* adhesion was not inhibited, but recall that *C. albicans* possesses multiple adhesins and the likelihood that a single 7-mer would inhibit all available adhesins is small. A 23-mer, GRGDSPASSKGGGSRLLLLLLR (S. A. Klotz, N. K. Gaur, J. M. Rauceo, and P. N. Lipke, Abstr., 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-375, p. 436, 2003), entirely inhibits the adherence of both *C. albicans* and *S. cerevisiae* expressing Als5p or Als1p to beads bearing any of the identified heptamers. However, the use of the 7-mer peptide library has allowed us to detect differences in ligand binding by two adhesins, Als5- and Als1p, a feature we would probably not have been able to detect if stronger binding peptides (such as the 23-mer) were used. Furthermore, the exogenous ligand need not be identical to the immobilized ligand for inhibition

TABLE 1. Peptide sequences to which fungi adhered<sup>a</sup>

Peptide-bead	Peptide sequence(s) <sup>b</sup>
Peptide-beads bound by <i>C. albicans</i> <sup>c</sup> .....	<b>KTKFLVD</b> , VTHTHRR, DKGWRAP, <b>KLRIPSV</b> , and <b>AYKSLMT</b>
Peptide-beads bound by <i>S. cerevisiae</i> expressing <i>ALS5</i> <sup>c</sup> .....	<b>FGYPIRR</b> , GHKNATR, PIANMRK, RHMAHKL, STGMKKM, and EFKSWRY
Peptide-beads bound by <i>S. cerevisiae</i> expressing <i>ALS1</i> .....	<b>HLYASWR</b> , VYYPFKQ, and <b>DLKLVRP</b>

<sup>a</sup> Fungi were mixed with ~10<sup>3</sup> beads from the 7-mer library.

<sup>b</sup> The sequence motif is indicated in boldface.

<sup>c</sup> These peptide sequences were confirmed.

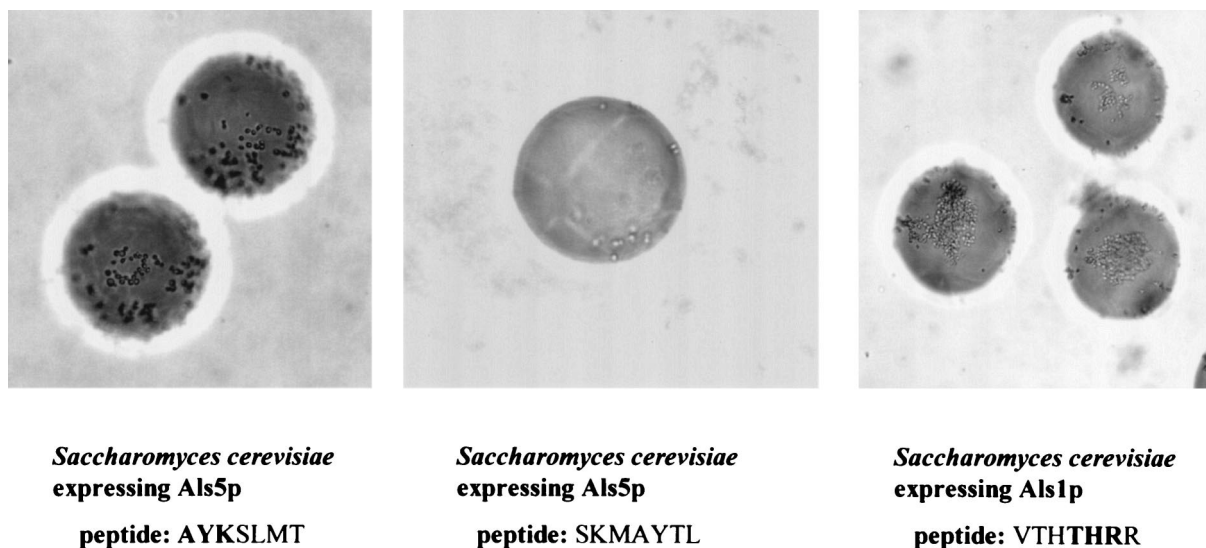


FIG. 2. (Left panel) *S. cerevisiae* expressing Als5p mixed with beads bearing AYKSLMT. All beads are covered by aggregating yeast cells. (Middle panel) *S. cerevisiae* expressing Als5p mixed with beads bearing SKMAYTL, the scrambled peptide of AYKSLMT. (Right panel) *S. cerevisiae* expressing Als1p adhering and aggregating on the surface of beads bearing the peptide, VTHTHRR. Note that the microcolonial phenotype is different from that of Als5p, since large patches of cells are adherent and aggregating on the surface of the bead.

of adherence to occur. For example, AYKSLMT and DKGWRAP, but not EHAHTPR (which does not have a consensus sequence), inhibited the adherence of *S. cerevisiae* expressing Als5p to beads bearing KLRIPSV (data not shown). The lack of requirement of an homologous peptide free in solution and on the bead underscores the degeneracy of the recognition system of these adhesins.

**Specificity of Als5p and Als1p.** To determine whether the two adhesins bound to the same ligands, the peptides originally identified as ligands for *C. albicans* were tested for their ability to be recognized by *S. cerevisiae* expressing either adhesin (Table 4). Cells expressing Als5p adhered to each of the ligand sequences. In contrast, *S. cerevisiae* cells expressing Als1p bound less well than Als5p to AYKSLMT and in even smaller numbers to VTHTHRR and not at all to three other adhesive peptides (KLRIPSV, KTKFLVD, and DKGWRAP). These results demonstrate that there is a clear difference in the ability of two Als proteins to recognize and adhere to various peptides. Furthermore, the adherence phenotype of these two adhesins is distinctly different. Als1p-bearing cells adhered initially in large clumps or aggregates of cells, whereas Als5p-bearing cells adhered initially as single cells (compare Fig. 2, left panel, with the right panel).

TABLE 2. Specificity of the adherence process demonstrated for the peptide sequence, AYKSLMT<sup>a</sup>

Peptide-bead	Specificity score			
	<i>C. albicans</i>	<i>S. cerevisiae</i> expressing <i>ALS5</i>	<i>S. cerevisiae</i> expressing <i>ALS1</i>	<i>S. cerevisiae</i>
AYKSLMT	+++	+++	+++	—
SKMAYTL	+++	—	—	—

<sup>a</sup> The same amino acids were then randomly assembled to prepare a "scrambled peptide sequence", SKMAYTL.

#### The binding sequence motif and its relationship to proteins.

The broad specificity of Als5p binding to such sequences as  $\tau\phi+$  and threonine, serine, and alanine patches (7) may explain previously observed characteristics of *C. albicans* binding to numerous proteins (11). *C. albicans* (or *S. cerevisiae* expressing an Als adhesin) binds better to many proteins after they are denatured such as disrupted basement membrane and denatured type I collagen known as gelatin (12, 14). BSA is another such example. Inspection of the sequence of BSA reveals 16  $\tau\phi+$  sequence motifs. At least 13 of these are buried in the native structure but would become exposed after denaturation. As a proof of this concept, BSA was denatured by boiling and added to *C. albicans* yeast cells, and this combination was compared to a similar number of yeast cells exposed to native BSA (Fig. 3). As indicated in the figure, there is adherence and aggregation around denatured BSA, whereas these are not detectable when native BSA is added. This same phenomenon occurs when BSA is immobilized on magnetic beads (data not shown), i.e., denatured BSA elicits adherence and aggregation, whereas native BSA does not. Similar results are obtained with *S. cerevisiae* expressing Als1 or Als5p (data not shown). Thus, denaturation exposes adhesive ligands such as  $\tau\phi+$

TABLE 3. Inhibition of Als5p with free ligand

Microorganism and adhesin	Inhibition score	
	Peptide-bead with VTHTHRR	Peptide-bead plus 500 $\mu$ g of free VTHTHRR/ml
<i>C. albicans</i>	+++	+++
<i>S. cerevisiae</i>	—	—
<i>S. cerevisiae</i> expressing Als5p	+++	—
<i>S. cerevisiae</i> expressing Als1p	++	++

TABLE 4. Adherence of fungi expressing different *ALS* genes to 7-mer peptides

Peptide-bead	Adherence score			
	<i>C. albicans</i>	<i>S. cerevisiae</i> expressing <i>ALS5</i>	<i>S. cerevisiae</i> expressing <i>ALS1</i>	<i>S. cerevisiae</i>
KTKFLVD	+	++	–	–
VTHTHRR	++	+++	+	–
DKGWRAP	++	+++	–	–
KLRIPSV	+++	+++	–	–
AYKSLMT	++	+++	++	–
EHAHTPR	–	–	–	–

and perhaps others, which elicit adherence followed by aggregation.

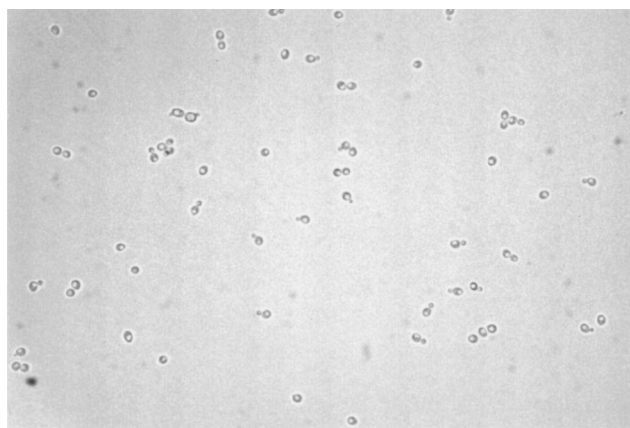
### DISCUSSION

This study is a logical outgrowth of previous work demonstrating the adherence of *C. albicans* and *S. cerevisiae* expressing *ALS5* or *ALS1* to immobilized peptides when they are presented in the appropriate steric configuration and composition (7). The use of a random peptide library as described herein clearly demonstrates that the recognition of peptide ligands by the Als proteins is degenerate, i.e., many peptides can be recognized and adhered to. This finding was suggested by our previous work (7) but could not easily be tested with the other peptide-beads since each peptide had to be individually synthesized and coupled to the beads—an extremely slow and inflexible method. The screening method used herein allows one to screen large numbers of beads but is not amenable to high throughput screening since the recognition system is degenerate, i.e., so many beads are bound by yeast cells.

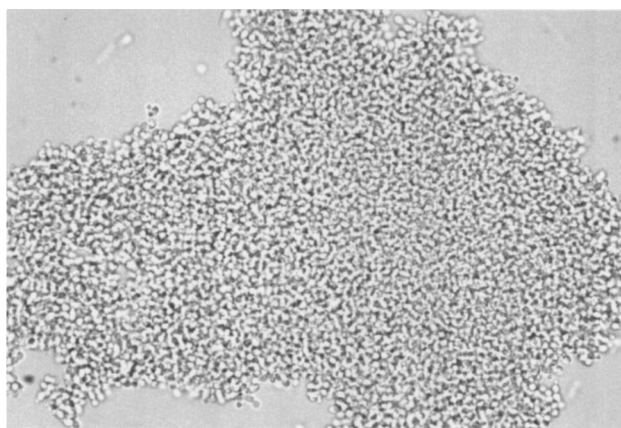
Adhesive peptides in the current study (Table 1) had several common properties. Basic residues were overrepresented rel-

ative to expectation from random composition (25 occurrences versus 11 expected by chance), and sequential basic residues were more common than expected (5 instances in 15 peptides versus 2 expected). There was a deficit in acidic residues (3 occurrences versus 11 expected), and they occurred only at the N terminus. Each peptide in Table 1, as well as the peptide APRLRFYSL mentioned previously, possesses the sequence motif. Note that the nonadherent control peptide EHAHTPR has no sequence motif. The scrambled peptide SKMAYTL, which is not adhesive, also has no sequence motif. A single naturally occurring adherent peptide without this sequence motif is known: FTTTSTSPV (7). Therefore, there must be additional binding specificity manifested by Als proteins in addition to the motif found in this work. Binding of  $\tau\phi$ + peptides could be competed and was specific. SKMAYTL, a “scrambled version of the adherent peptide,” AYKSLMT, was inactive with Als5p- and Als1p-bearing *S. cerevisiae* cells. The specificity of the interaction was further demonstrated by the fact that free peptide ligand inhibited the ability of *S. cerevisiae* expressing Als5p to adhere to the identical sequence on the surface of a bead (Table 3).

It should be borne in mind that *C. albicans* has many types of adhesins on the cell surface at any one time, including hydrophobic proteins (18), cell wall proteins that can be linked to human cells by host transglutaminase (19), and numerous Als proteins (9). This could explain why a greater fraction of library beads (>10-fold more) were coated by *C. albicans* than by *S. cerevisiae* expressing Als5p or Als1p. It also could explain why *C. albicans* adheres to sequences chosen by Als5p and Als1p and even “scrambled” sequences (Table 2). Furthermore, because *C. albicans* has potentially more adhesins present on the cell surface, it would explain why a soluble peptide, VTHTHRR (Table 3), inhibited *S. cerevisiae* expressing Als5p from adhering to beads bearing the homologous pep-



*Candida albicans*  
with native albumin



*Candida albicans*  
with denatured albumin

FIG. 3. Photomicrographs of *C. albicans* yeast cells mixed with BSA in native conformation (left panel) or denatured by boiling (right panel). Note that the cells on the left are present in singlets and doublets, demonstrating little or no interaction with BSA, whereas those on the right are aggregated into a large mass.

tide, whereas *C. albicans* could still adhere by using other Als adhesins. Only a larger peptide, a 23-mer, among peptides we have worked with inhibits *C. albicans* adherence to target proteins and peptides (15; Klotz et al., unpublished). Lastly, it must be appreciated that Als1p on the surface of *C. albicans* is not at its greatest magnitude because the cells were harvested in the stationary phase of growth, and this will affect the adherence of this fungus (3, 7a).

As mentioned above, yeast cell adherence to 7-mer peptide-coated beads is reversed upon the addition of formamide. Similarly, yeast cells blocked from adhering to beads by the addition of free peptides (such as VTHTHRR) when treated with formamide release the peptide-yeast cell interaction, and yeast cells can once again adhere to the peptide-coated beads (data not shown). This likely is occurring because the formamide disrupts the hydrogen bonds formed with the exposed peptide backbone of the target (7; Gaur and Klotz, unpublished). In this assay, neither nonionic detergents nor saccharides had an effect on adherence; therefore, a hydrophobic or lectin effect is not the sole mode of adhesive interaction in Als binding to peptides (5–7).

Hawser and Islam (8) measured the adherence of *C. albicans* to single amino acids immobilized in plastic wells and showed specific adherence to several amino acids and not to others. These authors also demonstrated the ability of BSA, when premixed with the yeast cells, to block the subsequent adherence to immobilized BSA but not to the amino acids. Our study is in concurrence with the findings with BSA but not that of the amino acids. This is very likely due to the nature of the assays used, since physical trapping, hydrophobic interactions, and probably other forces contribute to assays in wells, whereas the use of beads has no confounding background interactions that are measured as adherence. Furthermore, the bead-yeast interaction is not affected to any measurable extent by the type of buffer used or by the addition of sugars or Tween 20.

Differential adherence of the Als adhesins has been noted previously (7; Chan et al., Abstr. Sixth Annu. Candida Candidiasis Meet.; N. V. Lucindo, D. C. Sheppard, S. G. Filler, J. E. Edwards, and M. Zhang, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol. 2002, abstr. F-21, p. 204, 2002; D. C. Sheppard, M. Zhang, A. S. Ibrahim, Y. Fu, S. G. Filler, and J. E. Edwards, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 385, p. 438, 2003). The data are consistent with the concept that each Als protein has a broad repertoire of preferred amino acid sequences, and these repertoires differ among the different Als proteins. Furthermore, as seen in Fig. 2 (compare Fig. 2, left panel, with Fig. 2, right panel), the adherence phenotypes of these two Als proteins are distinctly different. The phenotype for Als1p can be explained by flocculation (3) or aggregation of the Als proteins (5).

The major finding in the present study relates to the wide range of peptide targets to which *C. albicans* can adhere. A degenerate “recognition system” among adhesins guarantees a plethora of target proteins for adherence. Such appears to be the case with *C. albicans*, which can bind to many proteins and peptides (11, 13, 14). Our previous study established that cells bearing Als5p adhere to every protein we studied provided the

peptide backbone was accessible for adherence (Gaur and Klotz, unpublished). Furthermore, denaturing proteins provides an adherence target for Als proteins by breaking down the secondary and tertiary structure of the protein and exposing previously buried  $\tau\phi+$  sequences and likely other sequences for recognition by Als proteins (Gaur and Klotz, unpublished).

#### ACKNOWLEDGMENTS

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