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Effect of Glutaraldehyde Fixation on Cell Surface Binding Capacity of *Candida albicans*

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The ability of viable and glutaraldehyde-fixed, stationary-phase yeast cells of *Candida albicans* to bind concanavalin A and monospecific antiserum for antigenic factor 1 was examined. Both fluorescence flow cytometric analysis and transmission electron microscopy indicated that glutaraldehyde-fixed cells bound less of the two reagents than did unfixed viable cells.

The surface of the dimorphic yeast *Candida albicans* is the initial site of physical contact between the organism and host both during normal colonization and during infection. Thus, changes in this surface may affect the host-parasite interaction. Studies have shown that there are yeast- and germ tube-specific surface components, including receptors for human proteins (4, 5, 7, 11, 14–16, 20). Analysis of hydrophobicity (9) and expression of the determinants of several monoclonal antibodies have suggested that the surface may be variable and that changes can occur in the absence of cell division (1–3, 7, 9, 10). We have recently reported other determinants recognized by monospecific antisera appear to be unaffected by the growth state or morphology of the organism (6). However, the extent of antigenic variability and the rapidity with which changes may be affected are unknown. A concern that viable cells would respond to their environment during the course of analysis with a change in cell surface topography has led us to examine cells which have been fixed to retain the cell surface topography. Glutaraldehyde is widely used in conjugating enzyme markers to antibodies and treatment of cells or tissues in preparation for histocytochemical procedures, since some biological properties such as enzyme activity and antigen-antibody recognition are often retained (8). We have examined the ability of viable and glutaraldehyde-fixed cells to bind the lectin concanavalin A and monospecific antiserum recognizing a mannan determinant.

Fluorescence flow cytometric analysis. Stationary-phase cells of *C. albicans* B311, serotype A, were washed, and some were held on ice or some were treated with 2% glutaraldehyde (grade 1; Sigma Chemical Co., St. Louis, Mo.) (6). Cells were incubated with fluorescein-conjugated concanavalin A (Sigma) in phosphate-buffered saline (PBS) (0.01 M phosphate [pH 7.4], 0.15 M NaCl) containing 1% bovine serum albumin for 30 min at room temperature and were washed four times with PBS. Maximum fluorescence was obtained at 200 μg/ml, and 300 to 500 μg/ml was used in subsequent assays. Concanavalin A conjugated with 15-nm gold particles (E-Y Laboratories, Inc., San Mateo, Calif.) was added to fluorescent conjugate at 5 μg/ml. The fluorescence of 10,000 cells was determined by using a fluorescence flow cytometer (FACStar; Becton Dickinson Immunocytometry Division, Mountain View, Calif.) (6). Monospecific rabbit antiserum for factor 1 (Iatron Laboratories, Tokyo, Japan) diluted 1:10 or normal rabbit serum diluted 1:25 in PBS containing 1% bovine serum albumin was incubated with cells, and subsequently, cells were incubated with fluorescein-conjugated goat anti-rabbit immunoglobulin diluted 1:10 in PBS containing 1% bovine serum albumin (6) or goat anti-rabbit immunoglobulin conjugated with 15-nm gold particles (E-Y Laboratories) diluted 1:10 in PBS containing 1% bovine serum albumin (6).

Fluorescence flow cytometric analysis showed that the fluorescence of control glutaraldehyde-fixed cells was greater than that of control viable cells (Fig. 1). Although all cells of both populations bound fluorescein-conjugated concanavalin A, the incremental increase in fluorescence intensity due to lectin binding of viable cells was greater than that of glutaraldehyde-fixed cells.

Factor 1 is one of several antigenic determinants present in the fine structure of yeast mannan, which has been identified in a serologic classification scheme (21). The ability of viable and glutaraldehyde-fixed cells to bind monospecific antiserum to this surface determinant was examined. Similar relationships to that observed with concanavalin A between control and antibody-treated cells of the two populations were obtained (data not shown). These experiments confirmed that cells of *C. albicans* fixed with glutaraldehyde retain the capacity to bind concanavalin A and antibody.

To determine whether the difference reflected decreased binding sites on glutaraldehyde-fixed cells or an unexpected failure to detect the same incremental increase over the elevated autofluorescence of fixed cells, both viable and fixed cells treated with reagents were prepared and examined by transmission electron microscopy.

Transmission electron microscopy. Cells were fixed for about 20 h at 4°C with 4% paraformaldehyde–1% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2 (13). Cells were washed three times in cacodylate buffer. Cells were treated for 60 min with 2% OsO_4 in cacodylate buffer and were washed three times for 15 min in water. Cells were stained for 60 min with saturated uranyl acetate, pH 3.3, rinsed with water, and dehydrated in a graded series of acetone for 45 min (17). Cells were embedded in Spurr low-viscosity resin and polymerized for 60 h at 60°C (19). Thick and thin sections were cut, and thin sections were stained with uranyl acetate, pH 3.3, for 15 min and then with lead for 10 min (18). Sections were examined on a Hitachi H-600 electron micro-

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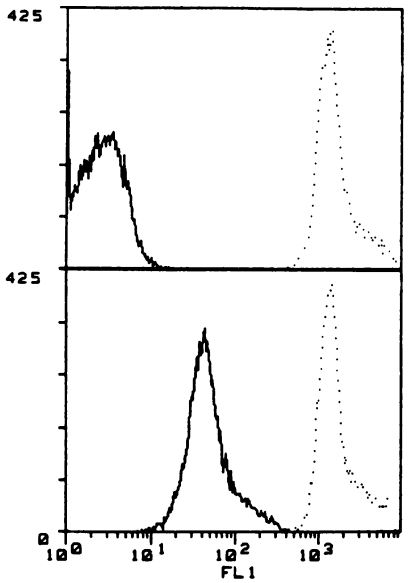

FIG. 1. Flow cytometric analysis of concanavalin A binding. Viable and glutaraldehyde-fixed cells were reacted with fluorescein-conjugated concanavalin A, as described in the text. The histograms in the top panel indicate viable cells, untreated control (——) and treated with concanavalin A (· · · ·), and the histograms in the lower panel indicate glutaraldehyde-fixed cells, untreated control (——) and treated with concanavalin A (· · · ·). The x axis indicates fluorescence intensity, and the y axis indicates the number of cells.

scope. Yeast cells were quantitated on electron micrographs on a digitizer (Ladd Research Industries, Burlington, Vt.). The total number of gold particles per unit length and the means and standard deviations were calculated. A minimum of 30 cells, in full cross-section, was analyzed for each experimental condition. Differences in means were compared at $\alpha = 0.05$ by a t test.

No differences in cell wall ultrastructure were observed, although the layers appeared more distinct in the cells fixed with glutaraldehyde before reaction with lectin or antibody (data not shown). Gold particles were present on the surface and bud scars as well as apparently embedded in the outer portion of the cell wall. No difference was observed in this distribution between the two cell preparations (data not shown). An analysis of the density of gold particles showed that cells fixed before reacting with lectin or antibody bound about 21% less concanavalin A and 39% less monospecific antiserum than did cells that had not been fixed prior to reaction (Table 1).

These experiments were unable to distinguish whether this difference in surface topography was qualitative as well as quantitative. Glutaraldehyde is considered to react primarily with ε-amino groups of lysine in proteins and perhaps glucosamine in fungal chitin (8). Since the two reagents recognize the specific fine structure of the mannan, it seems unlikely that the reduction in binding capacity resulted from covalent modification or cross-linking of the recognition sites. An alternative is reduction in accessibility of binding sites. The number of reagents used is too small to eliminate the possibility that the binding of other reagents could be unchanged or enhanced in fixed cells. The same considerations apply for formaldehyde-fixed cells, where binding of complement factor C3 fragments was unchanged (11) and binding of fibrinogen was reduced (16). Since glutaraldehyde is a common fixative for cells used in various analyses such as adherence and, particularly, in electron microscopy where binding reactions may be carried out on thin sections, the extent and localization of reactivity must be interpreted carefully. With *C. albicans*, the use of fixatives to preserve cell surface topography may be of as much concern as the potential of the viable cells to change the topography during analysis. Since bound ligands may stabilize a conformation, it is possible that the in vivo binding of one host protein may affect the interaction of *C. albicans* with other host proteins or cells by making binding sites more or less available.

TABLE 1. Binding of concanavalin A and factor 1 antiserum to viable and glutaraldehyde-fixed cells[a]

Treatment[b]	Gold particles (mean ± SEM)/μm of cell surface		% Reduction[c] of glutaraldehyde-fixed cells
	Viable	Glutaraldehyde fixed	
None	None	None	
Concanavalin A	2.44 ± 0.70	1.92 ± 0.48	21
Goat anti-rabbit immunoglobulin	None	None	
Normal rabbit serum + goat anti-rabbit immunoglobulin	0.47 ± 0.63	0.58 ± 0.50	NS
Factor 1 antiserum + goat anti-rabbit immunoglobulin	51.87 ± 22.84	31.70 ± 6.66	39

[a] Viable and glutaraldehyde-fixed cells were treated as indicated in the text and then were prepared for transmission electron microscopy, which involved a fixation step.

[b] Cells were treated with a mixture of fluorescein isothiocyanate- and colloidal gold-conjugated concanavalin A or colloidal gold-conjugated goat anti-rabbit immunoglobulin, as described in the text.

[c] Differences in means between viable and glutaraldehyde-fixed cells were compared at $\alpha = 0.05$. The reduction in binding is given for those which were significantly different. NS, No significant difference.

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