Adherence of *Candida albicans* to human salivary components adsorbed to hydroxylapatite

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Colonization of the oral cavity by *Candida albicans* involves adherence of yeast cells to oral surfaces. An assay was developed to measure the attachment of *C. albicans* cells, metabolically labelled with [³⁵S]methionine, to saliva-coated hydroxylapatite (SHA) beads – a model for the tooth surface. Using this assay approximately 1.26×10^6 *C. albicans* cells (50.5% of input cells) attached to the SHA beads (12 mg). Different strains of *C. albicans* adhered to varying degrees to SHA beads, but in general adherence was promoted by growth of cells at 28 °C and by starvation of cells for glucose. Proteins in human whole, or parotid, saliva samples were fractionated by gel filtration (Sephacryl S-200 column) and fractions were adsorbed to hydroxylapatite beads. Fractions that contained proline-rich proteins or statherin promoted attachment of *C. albicans* ATCC 10261 cells. Neuraminidase treatment of SHA beads, but not of cells, significantly increased yeast cell binding to the SHA beads. Neuraminidase activity in the oral cavity may unmask glycoprotein receptors involved in the adhesion of *C. albicans* to saliva-coated surfaces.

Keywords: *Candida albicans*, adherence, saliva, hydroxylapatite

INTRODUCTION

The yeast *Candida albicans* is a human commensal that is also an opportunistic pathogen. Between 18 and 40% of individuals carry *C. albicans* in their oral cavity (Odds, 1988), and although predominantly associated with the dorsum of the tongue, *C. albicans* can be cultured from saliva, and from swabs of cheek and tooth surfaces (Arendorf & Walker, 1980). *C. albicans* and other *Candida* species can cause a number of oral diseases, including pseudomembranous candidosis, atrophic candidosis, mucocutaneous candidosis, denture stomatitis and angular cheilitis. In addition, *Candida* species have been shown to be components of the subgingival microbiota of adults with periodontitis (Slots *et al.*, 1988; Rams & Slots, 1991) and *C. albicans* cells are often found in dental plaque (Arendorf & Walker, 1980). Despite the association of *Candida* with periodontitis and plaque there has been little study of the interaction between *Candida* and saliva-coated tooth surfaces.

Colonization of the human oral cavity by *C. albicans* is believed to be mediated through the ability of yeast cells to adhere to the variety of substrates present, including glycoconjugates on oral epithelial cells (Brassart *et al.*, 1991; Douglas, 1992; Tosh & Douglas, 1992), and to oral bacteria (Jenkinson *et al.*, 1990). Since most surfaces in the oral cavity are bathed in saliva, the binding of yeast cells to salivary proteins is of paramount significance in colonization. Some salivary proteins such as histatins (Lal *et al.*, 1992) and lysozyme (Togbi *et al.*, 1988) are inhibitory to yeast growth, while secretory immunoglobulin A (Vudhichamnong *et al.*, 1982) and mucins (Hoffman & Haidaris, 1993), which are bound by *Candida*, promote aggregation and assist clearance of yeast cells. However, when salivary components are adsorbed to surfaces they can act as ligands for *C. albicans* adhesion (Calderone & Braun, 1991; Edgerton *et al.*, 1993; Nikawa *et al.*, 1993). Proteins from saliva adsorb readily to oral surfaces, especially to tooth enamel, to form the acquired pellicle (Lamkin & Oppenheim, 1993). The specific components of the human salivary pellicle that support adherence of *C. albicans* have not been identified. Although there is some evidence that both high- and low-molecular-mass salivary mucins are involved in adherence of *C. albicans* to salivary pellicle formed on denture acrylic material (Edgerton *et*

Abbreviations: BEC, buccal epithelial cell; NeuNAc, *N*-acetylneuraminic acid; PRPs, proline-rich proteins; SHA, saliva-coated hydroxylapatite; SPMMA, saliva-coated polymethylmethacrylate.

al., 1993), purified mucins do not support adherence (Edgerton *et al.*, 1993; Nikawa *et al.*, 1993). In this paper we describe an assay we have developed to measure adherence of *C. albicans* cells to saliva-coated hydroxylapatite (calcium phosphate) beads (a model for the tooth surface). In addition we have used this assay to characterize further the interactions of *C. albicans* with salivary components.

METHODS

Yeast strains and culture conditions. The following *C. albicans* strains were used: ATCC 10261; CA2 and A72 (from A. Cassone, Istituto Superiore di Sanita, Rome, Italy); SGY-243 (*ade2/ade2 Δura3::ADE2/Δura3::ADE2*; Squibb Institute for Medical Research, Princeton, NJ, USA); hp11an and HMHC12 (human isolates, kindly provided by J. Schmid, Massey University, New Zealand). The yeasts were maintained in YPD (1%, w/v, yeast extract; 2%, w/v, Bacto-Peptone; 2%, w/v, glucose) containing 50% (v/v) glycerol at -80 °C. They were routinely cultured at 28 °C in a glucose/salts/biotin medium (GSB) containing (g l⁻¹) glucose, 10; (NH₄)₂SO₄, 1.0; KH₂PO₄, 2.0; MgSO₄·7H₂O, 0.05; CaCl₂·2H₂O, 0.05; and biotin, 0.05 mg l⁻¹ (Holmes *et al.*, 1991).

Radiolabelling of *Candida albicans* with [³⁵S]methionine. Yeast cells were grown in GSB medium at 28 °C or 37 °C with shaking for 16 h, and used to inoculate 20 ml cultures of GSB to give an OD₅₄₀ of 0.2. [³⁵S]Methionine (0.62 MBq, 17 μCi, 1 × 10³ Ci mmol⁻¹) was added to the cultures, which were incubated at 28 °C or 37 °C until the OD₅₄₀ reached 1.0. The cells were harvested by centrifuging (3000 g, 5 min), washed twice in 5 ml KCl buffer (2 mM KH₂PO₄/K₂HPO₄; 5 mM KCl; 1 mM CaCl₂, pH 6.5) and then resuspended in KCl buffer at a density of 2.5 × 10⁸ cells ml⁻¹. Uptake of radioactivity from the medium ranged from 76.5 to 90.7%, with a mean of 84.5 ± 4.0% (s.d.). The specific radioactivity of the cells (c.p.m. per cell) was calculated by measuring the radioactivity of the cell suspension and using a standard curve relating OD₅₄₀ to cell concentration. The specific radioactivity of the cell suspensions varied between 27 and 59 cells (c.p.m.)⁻¹ with a mean value of 38 ± 9 cells (c.p.m.)⁻¹. To determine the effect of glucose starvation on adherence, freshly labelled cells (20 ml, OD₅₄₀ = 1.0) were harvested by centrifuging (3000 g, 5 min), washed once in GSB without glucose, and suspended in GSB without glucose at an OD₅₄₀ of 1.0. The cells were incubated with shaking at 28 °C or 37 °C for 16 h, and were then harvested and washed in KCl buffer as described above.

Saliva-coated hydroxylapatite bead adherence assay. Saliva (unstimulated) was collected on ice from five donors and an equal amount from each donor was pooled. The saliva was clarified by centrifugation at 12000 g for 15 min, and then mixed with an equal volume of KCl buffer. Portions of hydroxylapatite beads (12 mg, BDH) were hydrated in 0.5 ml KCl buffer at 4 °C for 16 h. The KCl buffer was aspirated from the hydrated beads and either 1 ml saliva (50%, v/v, in KCl buffer), or 1 ml chromatography fraction (20%, v/v, in KCl buffer), was added. The tubes were mixed end-over-end (6–12 r.p.m.) at 22 °C for 60 min. The liquid was aspirated and the beads were washed three times with KCl buffer (1 ml each time). Additional protein-binding sites on the beads were blocked using a modification of the method of Gibbons & Etherden (1985). Beads were incubated with 1 ml KCl buffer containing 0.1% (w/v) bovine serum albumin (BSA) with end-over-end mixing at 22 °C for 1 h. The beads were washed once with KCl buffer (1 ml) and then 0.9 ml KCl buffer and 0.1 ml radiolabelled cells (diluted in

KCl buffer to the appropriate concentration) were added to each tube. The tubes were incubated at 22 °C with end-over-end mixing for 90 min. The liquid containing unattached cells was aspirated and then the beads were washed three times with KCl buffer (1 ml). The beads were transferred to scintillation vials and counted for radioactivity as previously described (Jenkinson *et al.*, 1990).

Neuraminidase treatment of SHA beads. In some assays BSA-blocked SHA beads were incubated with neuraminidase (10 mU, from *Vibrio cholerae*; Boehringer) in acetate buffer (50 mM, pH 6.0; 1 ml) with end-over-end mixing at 37 °C for 3 h. The beads were then washed once with KCl buffer and used in attachment assays as described above.

Neuraminidase assay and determination of *N*-acetylneuraminic acid (NeuNAc). The neuraminidase activity associated with *C. albicans* cells was measured by incubating 2.5 × 10⁷ cells with either fetuin (Sigma, 0.5 mg) or clarified whole saliva (200 μl) in a total volume of 600 μl 50 mM acetate buffer (pH 6.0) with end-over-end mixing at 37 °C for 1 h. The amount of NeuNAc released by the neuraminidase was determined using a thiobarbituric acid assay as described by Aminoff (1961) with NeuNAc (Sigma) as standard.

Column chromatography of saliva. Whole saliva (30 ml, stimulated by chewing parafilm) was collected from an individual subject, and in another experiment 20 ml parotid saliva (stimulated with an acid-drop sweet) was collected from the same subject using a modified Carlson-Crittenden device (Shannon *et al.*, 1962). The saliva samples were centrifuged twice (12000 g at 4 °C for 15 min) and proteinase inhibitors were added to 10 ml supernatant to give the following final concentrations: 1 mM phenylmethylsulphonyl fluoride, 10 mM bisulphite, 2 mM benzamidine hydrochloride, 3 μM pepstatin, 1 mM leupeptin, and 0.3 μM aprotinin.

Saliva (10 ml) containing proteinase inhibitors was applied to a Sephacryl S-200 (Pharmacia) column (94 × 2.6 cm), pre-equilibrated with 20 mM Tris/HCl (pH 8.0) containing 0.5 M NaCl, and eluted at 4 °C with the same buffer at a rate of 0.17 ml min⁻¹. The absorbance of the eluant was continuously monitored at 226 nm, and 3 ml fractions were collected and stored at 4 °C. Saliva samples and fractions were subjected to SDS-PAGE through 10% or 14% (w/v) acrylamide gels (Laemmli & Favre, 1973) and polypeptides were stained with silver nitrate (Morrissey, 1981).

RESULTS

Development of adherence assay

C. albicans ATCC 10261 cells adhered with high affinity to SHA beads and there was a linear relationship, up to an input of 2 × 10⁷ cells, between the number of *C. albicans* cells that adhered to the SHA beads (12 mg) and the number of yeast cells added to the assay (Fig. 1). At higher cell inputs the number of yeast cells adhering to SHA beads began to plateau, indicating that binding sites on the beads were becoming saturated. Multiple washings of the beads with KCl buffer did not significantly reduce the numbers of *C. albicans* cells attached. Scanning electron micrographs of SHA beads with attached cells showed that the yeast cells bound individually, in pairs, or in small clumps, and the cells were generally well-dispersed over the bead surface. The dimensions of 130 beads were measured from electron micrographs and it was estimated

Fig. 1. Binding of *C. albicans* ATCC 10261 to hydroxylapatite beads (12 mg) coated with whole saliva (●) or with bovine serum albumin (○) as a function of the number of cells added to the assay. Points represent the means of at least three determinations carried out on separate batches of cells. Error bars are $\pm$ SD.

Table 1. Effect of growth conditions on adherence of *C. albicans* to hydroxylapatite beads coated with human whole saliva

A total of 2.5×10^6 cells were added to assays containing 12 mg SHA beads. Results are the means of at least three separate determinations carried out on separate batches of cells.

Growth conditions	$10^{-5} \times$ No. of cells adhering to SHA beads ($\pm$ SD)	
	<i>C. albicans</i> ATCC 10261	<i>C. albicans</i> CA2
28 °C, Exponential phase	13.05 ± 0.70	6.46 ± 0.84
37 °C, Exponential phase	1.97 ± 0.41	4.85 ± 0.57
28 °C, Starved for glucose*	ND	8.35 ± 0.48
37 °C, Starved for glucose	0.93 ± 0.09	3.70 ± 0.76

ND, Not determined; cells clumped as described in the text.

* Incubation in GSB without glucose at 37 °C for 16 h.

that there were on average $4.8 \times 10^3 \pm 6 \times 10^2$ beads in each assay (12.8 ± 1.6 mg beads), with a total surface area of approximately 4.2 ± 1.4 cm². In a standard assay, yeast cells (2.5×10^6) were incubated with SHA beads at ratio of about 520 cells per bead, and on average 50.5 ± 6.2 % of input cells bound to the beads. In control assays, where beads were blocked with BSA but not coated with saliva, on average 1.20 ± 0.07 % input cells bound to beads. A slightly higher proportion of input cells (6.6 ± 0.3 %) bound to untreated, hydrated, beads.

There was saliva-dependent variation in the binding of *C. albicans* ATCC 10261 cells to SHA beads. The percentage of input cells that adhered to hydroxylapatite beads coated with whole saliva samples varied between 20.4 ± 1.5 %

Table 2. Attachment of *C. albicans* strains to SHA beads

Yeast cells were grown in GSB at 28 °C. Results are the means of at least three determinations carried out on separate batches of cells. The number of input cells was 2.5×10^6 .

<i>C. albicans</i> strain	$10^{-5} \times$ No. of cells adhering ($\pm$ SD)	Percentage of input cells adhering ($\pm$ SD)
ATCC 10261	12.60 ± 1.55	50.5 ± 6.2
CA2	5.78 ± 0.27	23.1 ± 1.1
A72	7.48 ± 1.29	29.9 ± 5.2
SGY-243	2.61 ± 0.58	10.4 ± 2.3
hp11an	13.80 ± 1.13	55.1 ± 4.5
HMHc12	3.10 ± 0.10	12.4 ± 0.4

Table 3. Effect of neuraminidase on *C. albicans* ATCC 10261 adherence

SHA beads or radiolabelled yeast cells were incubated with neuraminidase (37 °C, 3 h) as described in Methods. Results are the means of four determinations carried out on separate batches of cells. The number of input cells was 2.5×10^6 .

Neuraminidase treatment	$10^{-5} \times$ No. of cells adhering ($\pm$ SD)	Percentage of input cells adhering ($\pm$ SD)
None*	9.64 ± 0.79	38.5 ± 3.2
Beads	14.18 ± 1.94	56.7 ± 7.4
Cells	10.12 ± 1.20	40.5 ± 4.8

* Control; SHA beads were incubated in buffer at 37 °C for 3 h without neuraminidase.

and 48.3 ± 1.3 % for the five donors. When these saliva samples were pooled, however, 50.0 ± 2.7 % of input cells bound to SHA beads. For this reason, and to reduce the effect of day-to-day variations, pooled saliva samples were routinely used.

Effect of growth conditions on adherence

The effect of yeast growth conditions on the adherence of cells to SHA beads was determined with *C. albicans* ATCC 10261 and with strain CA2, which unlike ATCC 10261 cannot be induced to form mycelia (Mattia *et al.*, 1982). Attachment of CA2 cells to SHA beads was affected less by growth conditions than attachment of ATCC 10261 cells (Table 1). Growth of both strains at 37 °C instead of at 28 °C reduced their binding to SHA beads (Table 1). This reduction in binding was found to be significant ($P < 0.005$) by the Student's *t*-test for exponential-phase

Fig. 2. Fractionation of human whole (a) or parotid (b) saliva, and adherence of *C. albicans* ATCC 10261 to hydroxylapatite beads coated with various fractions. —, A_{226} of material eluted from Sephacryl S-200 column (peaks are designated I to X); ●, numbers of *C. albicans* cells adhered to hydroxylapatite beads coated with proteins from certain fractions (mean of three separate determinations carried out on samples from one fractionation $\pm$ SD).

ATCC 10261 cells and starved CA2 cells. Starvation of cells for glucose at 37 °C reduced attachment of cells of both strains to SHA beads; the reduced attachment for ATCC 10261 cells was significant at $P < 0.05$ (Student's *t*-test). However, when cells of strain CA2 were grown and starved at 28 °C, an increased amount of binding was observed (Table 1). Cells of strain ATCC 10261 clumped during glucose starvation at 28 °C and could not therefore be used in adherence assays.

Strain variation in adherence

The adherence of exponential-phase cells of several *C. albicans* strains, grown in GSB at 28 °C, to SHA beads was compared (Table 2). All six strains adhered to SHA beads, but to varying extents, ranging from 10.4 % of input cells for *C. albicans* SGY-243 to 55.1 % of input cells for *C. albicans* hp11an (Table 2).

Effect of neuraminidase on *C. albicans* adherence to SHA beads

Neuraminidase treatment of SHA beads increased adherence of *C. albicans* ATCC 10261 cells to beads by 47 % (Table 3). This increase in adherence was found to be significant ($P < 0.005$) by the Student's *t*-test. Neuraminidase treatment of SHA beads under these conditions resulted in the release of 1.68 ± 0.06 μ g NeuNAc from 12 mg SHA beads. However, treatment of cells with neuraminidase had no significant effect on subsequent adherence to SHA beads (Table 3). To investigate the

Fig. 3. SDS-PAGE of proteins (stained with silver nitrate) in peak samples from whole saliva fractionation. Samples (20 μ l) of fractions 5–88 were separated on a 10% (w/v) gel and fractions 108 and 128 were separated on a 14% (w/v) gel. Numbering indicates the following polypeptides (see Strömberg et al., 1992): 1, mucins; 2, heavy chain of secretory immunoglobulin A; 3, proline-rich glycoproteins; 4, proline-rich proteins (PRPs); 5, statherin; 6, α -amylase.

possibility that variation in adherence between *C. albicans* strains was due to strain-specific neuraminidase production, the neuraminidase activity associated with *C. albicans* cells grown as for the adherence assay was measured using fetuin or whole saliva as substrate. No release of NeuNAc was detected for any *C. albicans* strain tested under conditions where commercial neuraminidase (10 mU) released between 12.7 µg and 14.8 µg NeuNAc.

Fractionation of human saliva

Whole or parotid saliva samples from an individual donor, whose saliva gave consistently high binding ($48.3 \pm 1.3\%$ input cells), were fractionated by column chromatography. The absorbance profiles for material eluted from the column are shown in Fig. 2. Samples (20 µl) of the peak fractions were subjected to SDS-PAGE and the proteins present were visualized by silver staining (Fig. 3). By comparison with the proteins fractionated from parotid saliva by Strömberg *et al.* (1992) the following salivary proteins were identified: 1, mucins; 2, heavy chain of secretory IgA; 3, proline-rich glycoproteins; 4, proline-rich proteins (PRPs); 5, statherin; 6, amylase (Fig. 3). The major differences between the profiles of parotid and whole saliva samples were that the parotid saliva contained a reduced proportion of mucins (peak I) and was enriched for proline-rich glycoproteins and proline-rich proteins (peaks II–IV).

Samples of the salivary fractions were used to coat hydroxylapatite beads and the numbers of yeast cells binding to these beads were measured (Fig. 2). Samples from peak III, IV or VIa fractions promoted adherence strongly, whereas samples from peak I, II, V, VIb or VII fractions supported little adherence. No attachment was observed with samples from peak VIII, IX or X fractions (Fig. 2). A component of peak VII fractions from parotid saliva was particularly effective in promoting adherence. Samples from peak III fractions containing PRPs promoted the greatest adherence of *C. albicans* cells.

DISCUSSION

Hydroxylapatite beads coated with salivary components have been used previously to measure the adherence of oral bacteria such as streptococci and actinomyces (Clark *et al.*, 1978; Eifert *et al.*, 1984; Strömberg *et al.*, 1992; Hawkins *et al.*, 1993). This is the first report of the use of hydroxylapatite beads to measure the adherence of *Candida* cells. Radiolabelling *C. albicans* cells to high specific activity enabled an accurate determination of adhered yeast cells. In the SHA bead assay an input ratio of approximately 520 *C. albicans* cells per bead was used, and on average 50.5% of input yeast cells adhered to SHA beads. This compares with between 0.3% cells binding, at an input ratio of 1000 *C. albicans* cells per buccal epithelial cell (BEC) (Douglas *et al.*, 1981), and 29.1% for an input ratio of 10 yeast per BEC (Sandin *et al.*, 1987) for BEC assays. Recently an assay was reported that measured attachment of *C. albicans* cells to saliva-coated polymethylmethacrylate (SPMMA; dental acrylic) beads

(Edgerton *et al.*, 1993). Using an input of 5×10^7 cells, at a ratio of 380 cells per bead, 1.9% of cells bound to the SPMMA beads. Clearly in the SHA bead assay adherence was demonstrated by a larger proportion of the input cells, suggesting a higher affinity of *C. albicans* for SHA beads compared to BECs or SPMMA beads.

Adherence of *C. albicans* cells was saliva-mediated, as negligible attachment was observed to uncoated beads or to beads incubated with BSA. The percentage of cells binding to beads coated with whole saliva was influenced greatly by cell growth conditions. These results were consistent with other observations of the effects of growth temperature and of glucose-starvation on the adhesive properties of *C. albicans*. For example, yeast cells grown at 28 °C were more adherent to BECs than were those grown at 37 °C (Kennedy & Sandin, 1988), and glucose starvation of yeast cells promoted their adherence to oral streptococci (Jenkinson *et al.*, 1990). Thus growth conditions also influence the expression of surface receptors on *Candida* involved in adhesion with saliva components. *Candida* adhesion was strain specific, which is also well documented (Jenkinson *et al.*, 1990; Tosh & Douglas, 1992). It is interesting to note that *C. albicans* SGY-243 (Ura⁻), a strain used widely for molecular genetic manipulations (Kelly *et al.*, 1987; Cannon *et al.*, 1992), adhered poorly to SHA beads.

Sephacryl S-200 chromatography of saliva resulted in the separation of salivary proteins into several discrete peaks. There are significant differences in the protein compositions of saliva samples from different individuals (Beeley *et al.*, 1991), and in this study attachment of *C. albicans* cells varied according to the source of the saliva. For the majority of experiments, problems associated with day-to-day and individual-to-individual variations in saliva samples were overcome by pooling samples from five individuals. However, to reduce the complexity of the starting material, fractionation of one individual's saliva, which promoted greatest attachment of *C. albicans* cells to HA beads, was carried out. Fractions of whole saliva containing PRPs (fractions 22–30, peaks III and IV) or statherin (fraction 66, peak VI) promoted *Candida* adhesion strongly (Fig. 2). The trend was similar with fractionated parotid saliva, although an unknown component of peak VII also promoted binding strongly.

It was initially reported that the mucin component of rat submandibular gland extract promoted adherence of *C. albicans* cells (Hoffman & Haidaris, 1993). However Hoffman & Haidaris (1994) have subsequently shown that the major *C. albicans*-binding moiety in the extract was a proteoglycan, denoted submandibular gland secreted proteoglycan 1, which contained carbohydrate structures found in heparan sulphate and chondroitin sulphate. Likewise, mucins, which are major constituents of pellicle formed on denture acrylic material *in vitro* (Edgerton *et al.*, 1993), are proposed to contribute receptors for adhesion of *C. albicans*, although purified mucins did not promote adherence (Edgerton *et al.*, 1993). Our data showed that saliva fractions containing mucins did not promote adhesion of *C. albicans* cells to HA beads.

In addition, early salivary pellicle formed on hydroxylapatite is thought to have a low content of low-molecular-mass mucins (Levine *et al.*, 1985). Therefore, as far as salivary pellicle on tooth surfaces is concerned, adherence of *C. albicans* to PRPs may be more significant, although our experiments do not rule out the possibility that *in vivo* a complex between mucins, PRPs and other proteins may mediate attachment.

Acidic PRPs are characterized by an abundance of proline, glutamic acid/glutamine and glycine, and they are involved in the inhibition of calcium phosphate crystal growth. Statherin also binds calcium ions and prevents calcium phosphate precipitation from saliva (Lamkin & Oppenheim, 1993). Both statherin and PRPs, bound to hydroxylapatite, have been shown to be receptors for the adherence of *Actinomyces naeslundii* (*viscosus*) (Gibbons & Hay, 1988) and PRPs act as receptors for the adhesion of *Streptococcus mutans* (Gibbons & Hay, 1989). The N-terminal regions of acidic PRPs, containing phosphoserine residues and other acidic amino acids, bind to hydroxylapatite (Lamkin & Oppenheim, 1993). This portion of the molecule is also involved in calcium binding and it is proposed that a conformational change induced in PRPs by attachment to hydroxylapatite unmasks the C-terminal region (a so-called cryptitope) which can then act as a receptor for bacterial cell adhesion (Gibbons & Hay, 1988). Our data suggest that *C. albicans*, like the aforementioned oral bacteria, may also recognize receptor components in the proline-rich protein fraction when these components are bound to hydroxylapatite.

Binding to PRPs could also be involved in adhesion of *C. albicans* to buccal or lingual epithelial cells. The surfaces of these cells adsorb salivary proteins, and the membrane-bound epithelial enzyme transglutaminase has been shown to covalently cross-link acidic PRPs to epithelial surface proteins (Bradway *et al.*, 1992). Thus binding of *C. albicans* to PRPs that are adsorbed or covalently-linked to epithelial cells may be the first step in a transglutaminase-catalysed mechanism of candidal adhesion that has recently been proposed (Bradway & Levine, 1993).

NeuNAc residues, which are present on a number of salivary glycoproteins, are thought to play a role in the adhesion of *Streptococcus sanguis* to saliva-coated surfaces by converting the initial association between bacteria and pellicle to a high-affinity binding (Cowan *et al.*, 1987). In contrast, attachment of *Actinomyces naeslundii* to human erythrocytes was promoted by neuraminidase treatment of the erythrocytes (Costello *et al.*, 1979), which was proposed to unmask galactose-containing receptors. It is significant that neuraminidase treatment of SHA beads increased the adherence of *C. albicans* cells. In the oral cavity, neuraminidase produced by bacteria may act on saliva-coated surfaces to unmask carbohydrate receptors, such as galactosyl or fucosyl residues to which *C. albicans* has been shown to bind (Brassart *et al.*, 1991; Tosh & Douglas, 1992).

Thus, in addition to adherence of *C. albicans* cells to specific salivary proteins, such as PRPs attached to oral surfaces, enzymic modification of salivary glycoproteins

may reveal other receptors that are involved in adherence, and hence colonization of the oral cavity by *C. albicans*.

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