Identification of salivary basic proline-rich proteins as receptors for *Candida albicans* adhesion

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The adherence of *Candida albicans* cells to oral surfaces is believed to be an important step in the development of oral candidosis. Electrophoretically separated parotid salivary proteins were transferred to nitrocellulose membranes and incubated with [³⁵S]methionine-radiolabelled *C. albicans* cells in a cell overlay adherence assay. A subset of four proteins with apparent molecular masses of 17, 20, 24 and 27 kDa (designated bands A–D) acted as receptors for cells of *C. albicans* ATCC 10261 and four clinical *C. albicans* isolates, in overlay assays. The N-terminal amino acid sequence of bands A–D indicated that these proteins were members of the basic proline-rich protein (bPRP) family. Digestion of protein A with endoproteinase Glu-C resulted in a single band (designated Ap) detected by Coomassie blue staining after SDS-PAGE. This band was not bound by *C. albicans* cells in overlay assays and comprised two fragments, designated ApN and ApC. These fragments had N-terminal sequences corresponding to the N-terminal and post endoproteinase Glu-C cleavage site sequences of bPRP IB-6 and had molecular masses of 6189 and 4261 Da as determined by mass spectrometry. Thus intact bPRP IB-6, and other bPRPs, may act as receptors for *C. albicans* adhesion.

Keywords: *Candida albicans*, basic proline-rich proteins (bPRPs), adherence, parotid salivary proteins

INTRODUCTION

The yeast *Candida albicans* forms part of the normal human oral microbial flora and can be isolated from the mouths of 20–40% of healthy individuals (Odds, 1988). Under conditions of host immunosuppression, *C. albicans* can cause a variety of mucosal infections, including pseudomembranous candidosis, erythematous candidosis, hyperplastic candidosis, *Candida*-associated denture stomatitis and *Candida*-associated angular cheilitis (Odds, 1988; Scully *et al.*, 1994). Indeed, oral candidosis is a prognostic indicator for AIDS, with 33–50% of HIV-seropositive and AIDS patients developing oral candidosis, which can lead to serious oesophageal infections (Samaranayake & Holmstrom, 1989; Samaranayake, 1992).

Oral surfaces are coated with salivary pellicles formed

by the selective adsorption of components from whole saliva and from other minor secretions, e.g. serum exudate (Sonju *et al.*, 1974). Pellicle maturation involves additional intermolecular protein interactions and enzymic modification, such as the covalent linking of proline-rich proteins (PRPs) to buccal epithelial cells (Bradway *et al.*, 1992). Proteinaceous pellicles provide a range of immobilized receptors for microbial adhesion (Jensen *et al.*, 1992). An initial step in the establishment of oral candidosis involves the adhesion of yeast cells to saliva-coated host tissues and so it is important to study the interaction between *Candida* cells and components of salivary pellicles. An understanding of the structure and function of the molecules involved in this interaction may enable the development of methods to prevent the adherence of *Candida*, or promote the clearance of *Candida* cells from the oral cavity.

C. albicans cells bind to a number of receptors, including fibronectin (Skerl *et al.*, 1984) and the H sugar sequence found on all blood group substances of the ABO(H) system (Brassart *et al.*, 1991). Adherence of *C. albicans*

Abbreviations: bPRP, basic proline-rich protein; PRG, proline-rich glycoprotein; PRP, proline-rich protein.

cells to a single component of human whole saliva, provisionally identified as low-molecular-mass mucin MG2, and to rat submandibular gland (RSMG) mucin (Hoffman & Haidaris, 1993) has also been demonstrated. However, it was later shown that purified mucins did not promote the adhesion of *C. albicans* to polymethylmethacrylate (Edgerton *et al.*, 1993) and that the *C. albicans*-binding component of RSMG was in fact a proteoglycan (Hoffman & Haidaris, 1994). Similarly, we have previously demonstrated that parotid saliva fractions containing mucins did not promote the adherence of *C. albicans* cells to hydroxylapatite beads (Cannon *et al.*, 1995). *C. albicans* cells were found to adhere to fractions of saliva containing PRPs, which have also been shown to promote adhesion of *Streptococcus gordonii* to apatitic surfaces (Gibbons *et al.*, 1991). The objective of this study was to further characterize components from parotid saliva that act as receptors for *C. albicans* adherence.

METHODS

Yeast strains, media and culture conditions. The following *C. albicans* strains were used in this study: ATCC 10261; I1 (hp11an; Schmid *et al.*, 1990); I17 (hp50an; Schmid *et al.*, 1995); I33 (HMHc4; Schmid *et al.*, 1990); and I44 (sw-17c; Schmid *et al.*, 1995). *C. albicans* cells were grown in glucose salts biotin medium (GSB; Holmes & Shepherd, 1988) at 30 °C with shaking (250 r.p.m.). Radiolabelled *C. albicans* cells were prepared by inoculating GSB (40 ml), containing 0.76 µCi (28.0 kBq) [³⁵S]methionine ml⁻¹, with stationary phase cells (1.1 × 10⁸ cells ml⁻¹) and incubating at 30 °C until the cell density reached 2.2 × 10⁷ cells ml⁻¹. Cells were harvested by centrifugation (6000 g, 15 min) and washed twice with 4 ml KCl buffer (2 mM KH₂PO₄, 2 mM K₂HPO₄, 5 mM KCl, 1 mM CaCl₂, pH 6.5) before being resuspended in KCl buffer at a concentration of 2.6 × 10⁸ cells ml⁻¹. [³⁵S]Methionine uptake from the medium varied between 80 and 96%, with a mean of 88.8 ± 6.6% (SD). The specific radioactivity of cells was calculated by measuring the radioactivity of a cell suspension and varied between 13 and 38 cells (c.p.m.)⁻¹ with a mean value of 17 ± 5.2 cells (c.p.m.)⁻¹.

Collection of parotid saliva. Stimulated parotid saliva (10 ml) was collected from donors, using a modified Carlson-Crittenden device (Shannon *et al.*, 1962), and stored on ice. Proteinase inhibitors were added to final concentrations of 1 mM PMSF, 10 mM bisulphite, 2 mM benzamidine.HCl, 2.9 mM pepstatin A and 0.3 mM aprotinin. The parotid saliva from six donors, three male and three female with a mean age of 37.5 years, was pooled and clarified by centrifugation (12000 g, 4 °C, 15 min).

Column fractionation of parotid saliva. Pooled parotid saliva (60 ml) was dialysed against water (18 h), lyophilized and resuspended in 10 ml Tris/NaCl elution buffer (20 mM Tris/HCl, pH 8.0; 0.5 M NaCl). The sample (92 mg protein) was then loaded onto a Sephacryl S200 (fractionation range from 5 to 250 kDa) chromatography column (XK 26/100; Pharmacia) and eluted with Tris/NaCl buffer (0.17 ml min⁻¹; Strömberg *et al.*, 1992). Column eluate was monitored at 226 nm (2138 Uvicord S; LKB), fractions (4 ml) were collected, and the peak fractions were pooled.

Hydroxylapatite-bead adhesion assay. The attachment of radiolabelled *C. albicans* cells to parotid-saliva-coated

hydroxylapatite beads (20 mg; Phase Sep) was measured as described by Cannon *et al.* (1995).

SDS-PAGE and electroblotting. SDS-PAGE was performed according to the method of Laemmli (1970) at 15 V cm⁻¹ and 16 °C. The apparent molecular mass of proteins was estimated using prestained protein markers (2.9–44 kDa and 14.7–200 kDa; Gibco-BRL). Sample protein concentrations were determined using a modified Bradford assay (Bio-Rad). Proteins were electroblotted (Towbin *et al.*, 1979) onto nitrocellulose membranes (Hybond-C) for use in blot overlays or onto PVDF membrane (Immobilon-P) for N-terminal amino acid sequencing.

Blot overlay. A modification of the method of Prakobphol *et al.* (1987) was used. Western-blotted proteins on nitrocellulose membranes were visualized by staining them with 0.2% Ponceau S in 1% (v/v) acetic acid for 5 min, followed by four washes with 1% (v/v) acetic acid. Ponceau S binds reversibly to protein and is reported not to alter epitopes on the immobilized proteins (Klein *et al.*, 1995). The membranes were destained with two washes (30 min, 20 °C) of PBS (8.5 g NaCl l⁻¹, 0.3 g KH₂PO₄ l⁻¹, 0.6 g Na₂HPO₄ l⁻¹, pH 7.5) and blocked with 5% (w/v) BSA in KCl buffer (2 h, 20 °C). Western blots were incubated with radiolabelled *C. albicans* cells [30 ml; 1.1 × 10⁷ cells (ml KCl buffer)⁻¹] for 18 h at 4 °C with reciprocal shaking (70 min⁻¹). Membranes were washed four times in PBS/0.1% Tween 20 to remove nonspecifically bound *C. albicans* cells and air-dried prior to exposure to X-ray film.

Protein analyses. Solid-phase protein samples on PVDF membranes were N-terminally microsequenced by automated Edman degradation (Hubbard, 1995).

Protease digestion was performed by the method of Cleveland *et al.* (1977). Proteins were separated on 16% polyacrylamide gels, stained with Coomassie blue, and portions of the gel containing specific bands were excised. These proteins were digested with 0.06–0.6 units endoproteinase Glu-C (sequencing grade; Promega). Digestion was performed within a polyacrylamide stacking gel, with no current for periods of 10 or 20 min, and the products were then separated by electrophoresis through a 16% polyacrylamide gel (25 V cm⁻¹, 22 °C).

Proteins were prepared for mass spectrometry (MS) by extraction from nitrocellulose with trifluoroacetic acid (TFA) and acetonitrile using the method of Harrington (1990). Samples were freeze-dried and resuspended in 5–10 µl 0.1% TFA, to a concentration of 5–10 pmol µl⁻¹. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS was performed using a Finnigan LaserMat 2000 MALDI-TOF mass analyser. The mass spectrometer was calibrated against the α-cyanohydroxycinnamic acid matrix peak at 379.4 Da.

RESULTS

Binding of *C. albicans* cells to electrophoretically separated parotid saliva proteins

Human parotid salivary proteins were separated by SDS-PAGE, electroblotted onto nitrocellulose membranes and incubated with radiolabelled *Candida* cells. *C. albicans* ATCC 10261 cells bound reproducibly to 16 proteins with molecular masses of between 17 and 63 kDa. There were a number of immobilized protein bands which did not stain intensely with Ponceau S but

Fig. 1. Comparison of *C. albicans* ATCC 10261 cell adherence to electrophoretically separated parotid salivary proteins with that of clinical strains I1, I17, I33 and I44. Parotid salivary proteins were separated on a 12.5% polyacrylamide gel and electroblotted onto nitrocellulose membranes. The membranes were incubated with 30 ml radiolabelled cells ($1.2\text{--}1.5 \times 10^7$ cells ml^{-1}). Experiments were performed at least three times. Representative results are shown. (a) Parotid saliva (15 μg) stained with Ponceau S. (b) Autoradiogram of parotid saliva Western blots (15 μg protein per lane) incubated with *C. albicans* ATCC 10261 (lane 1), *C. albicans* I1 (lane 2), *C. albicans* I44 (lane 3), *C. albicans* I17 (lane 4) and *C. albicans* I33 (lane 5). Arrowheads indicate the subset of proteins to which all *Candida* strains tested bound.

that were bound avidly by *C. albicans* cells (Fig. 1). Conversely, several protein bands that stained intensely with Ponceau S did not act as receptors for *C. albicans* ATCC 10261 cell adherence.

A subset of four parotid salivary protein bands with molecular masses of 17 (band A), 20 (band B), 24 (band C) and 27 (band D) kDa (Fig. 1) promoted the adherence of *C. albicans* ATCC 10261 cells, even at low concentrations of parotid salivary protein (2 μg per lane). The binding of various *C. albicans* clinical isolates to parotid salivary proteins was compared. There was strain-specific variation in the proteins to which cells adhered and in the proportion of input cells adhering to certain protein bands (Fig. 1). However, all strains tested bound to bands A–D (Fig. 1), and so these proteins were investigated further. Comparisons of the molecular masses of proteins A–D with the published migration positions of the major human parotid salivary proteins (Beeley *et al.*, 1991) indicated that they may be basic PRPs (bPRPs; molecular masses of 17 and 20 kDa), acidic PRP A (27 kDa) and an unspecified PRP (24 kDa).

In the hydroxylapatite-bead adhesion assay, a greater proportion of clinical isolate I1 cells bound to saliva-coated hydroxylapatite beads than did ATCC 10261

Table 1. Adherence of *C. albicans* strains to saliva-coated hydroxylapatite beads

Yeast cells were grown in GSB at 30 °C. Results are the means of at least six determinations carried out on a representative batch of cells. The number of input cells was $2.40 \pm 0.39 \times 10^6$ (mean $\pm$ SD).

<i>C. albicans</i> strain	$10^{-5} \times$ No. of cells adhering ($\pm$ SD)	Adherence relative to <i>C. albicans</i> ATCC 10261 (%)
ATCC 10261	3.66 ± 0.59	100
I1	5.07 ± 0.42	112.5 ± 9
I17	1.95 ± 0.42	53.4 ± 1.15
I33	0.53 ± 0.07	13.8 ± 1.6
I44	2.34 ± 0.38	87.5 ± 13.8

Fig. 2. Preparative fractionation of parotid saliva on a Sephacryl S200 column. The elution profile (–) is expressed as the percentage maximum A_{226} deflection; the thick bar denotes pooled fractions. Pools 1–3 contain the high-molecular-mass components and PRGs, pools 4 and 5 contain PRPs, pools 7 and 8 contain unknown components, pool 9 contains statherin and pool 12 contains mainly α -amylase (Strömberg *et al.*, 1992).

cells (Table 1). The proportion of strain I44 cells binding to beads was slightly less than that for ATCC 10261 and the proportion of adherent cells was markedly decreased for strains I17 and I33. Interestingly, the amount of cell binding to proteins on the overlay assay (Fig. 1) correlated with the adherence measured using the saliva-coated hydroxylapatite-bead adhesion assay. All strains were labelled with [^{35}S]methionine to the same specific radioactivity.

Fractionation of parotid saliva

Parotid saliva was fractionated by column chromatography (Fig. 2) using a modification of the method of Strömberg *et al.* (1992). Fractions containing protein peaks, judged by the A_{226} , were pooled (Fig. 2). The

Fig. 3. Binding of *C. albicans* ATCC 10261 cells to fractionated parotid salivary proteins. Proteins in fraction pools (Fig. 2) were separated on a 12.5% polyacrylamide gel and electroblotted onto a nitrocellulose membrane. The protein loading was 15 µg protein per lane. (a) Ponceau-S-stained nitrocellulose blot. Lanes 1–5 are pools 1–5, respectively. (b) Autoradiogram of the membrane in (a) incubated with *C. albicans* ATCC 10261 cells. Lanes are the same as (a). Arrowheads indicate the subset of proteins to which all *Candida* strains tested bound.

proteins present in these fraction pools were concentrated by dialysis against NH_4HCO_3 , lyophilized and resuspended in KCl buffer. The protein-banding pattern of the pooled parotid salivary proteins after SDS-PAGE and Ponceau S staining was not affected by concentrating the pools. Pools 1–4 each contained several proteins with a wide range of molecular masses (Fig. 3). This may indicate that under the nondenaturing chromatography conditions, proteins formed hetero-complexes which were subsequently resolved into component proteins by SDS-PAGE. Comparisons of the molecular masses of the electrophoretically separated proteins suggested that pools 1–4 contained proline-rich glycoproteins (PRGs) and PRPs (Strömberg *et al.*, 1992). Hydroxylapatite-bead adhesion assays confirmed that pools 1–4 promoted the highest levels of *C. albicans* adherence. Blot overlay assays carried out on the fraction pools indicated that most of the binding of *C. albicans* ATCC 10261 cells occurred to proteins present in pools 2, 3 and 4 (Fig. 3). The *C. albicans* cells bound to the same subset of proteins identified in overlay assays using unfractionated saliva (Fig. 1). Pools 6–12 contained very few proteins and gave a similar banding pattern in the overlay assay to pool 5, except that there was no binding to bands A or B (data not shown).

Characterization of bands A, B, C and D

Bands A and B were electrophoretically separated from pool 3 and bands C and D were separated from parotid saliva. They were then transferred to PVDF membrane (Towbin *et al.*, 1979) and excised. Approximately 180 pmol band A and band B was sequenced, and each was found to have the same N-terminal amino acid sequence, $\text{NH}_3\text{-SPPGKPQGPPQGGNQPQGP}$, with no indication of a contaminating sequence. Bands C and D had the same N-terminal sequence, $\text{NH}_3\text{-SPPGKPQG}$. A comparison of the N-terminal sequence of bands A and B with protein databases resulted in five matches

Table 2. Proteins with the same N-terminal amino acid sequence as bands A and B

Proteins that showed 100% sequence similarity to A and B over the initial 20 N-terminal amino acids are listed. Matches were determined using the BLAST algorithm (Altschul *et al.*, 1990).

Protein identity	Predicted molecular mass* (Da)	Predicted endoproteinase Glu-C digestion products (Da)
IB-6 (Kauffman <i>et al.</i> , 1986)	11 518	7274; 4262
IB-7 (Kauffman <i>et al.</i> , 1991)	5770	5285; 503
IB-8a (Kauffman <i>et al.</i> , 1991)	12 430	5295; 5285; 1886
IB-8c (Kauffman <i>et al.</i> , 1991)	5843	5843
IB-9 (Kauffman <i>et al.</i> , 1982)	6025	5313; 730

* Molecular mass was calculated using the GeneJockey program (Biosoft; Cambridge).

with 100% similarity over the 20 amino acids (Table 2). All matches were with human parotid salivary non-glycosylated bPRPs. As these proteins (IB-6, IB-7, IB-8a, IB-8c and IB-9) each have the same N-terminal sequence (Kauffman *et al.*, 1991), identification of the proteins in bands A, B, C and D beyond being members of the nonglycosylated bPRP family could not be made at the level of N-terminal amino acid sequence.

Band A was chosen for further analysis as it was the smallest member of the parotid salivary protein subset to which *C. albicans* cells bound. Endoproteinase Glu-C (V8 proteinase) cleaves proteins on the carboxyl side of glutamic acid or aspartic acid residues. The predicted endoproteinase Glu-C digestion products for IB-6, IB-7, IB-8a, IB-8c and IB-9 are given in Table 2. Endoproteinase Glu-C digestion of band A produced a single

Fig. 4. Endoproteinase Glu-C digestion product of band A. Band A was subjected to endoproteinase Glu-C digestion and then electrophoresis through a 16% polyacrylamide gel. The gel was stained with Coomassie blue by the method of Cleveland *et al.* (1977). Lanes: 1, low-molecular-mass markers; 2, band A; 3, band A plus 0.6 units endoproteinase Glu-C; 4, band A plus 0.3 units endoproteinase Glu-C; 5, band A plus 0.06 units endoproteinase Glu-C; 6, 0.6 units endoproteinase Glu-C. A, Band A; Ap, endoproteinase Glu-C digestion product of band A.

product band (Ap) detected by Coomassie blue staining (Fig. 4). Ap had an apparent molecular mass of 7.4 kDa, compared with an apparent molecular mass of 15.2 kDa for band A. Amino acid sequencing of protein band Ap indicated that there were two predominant peptides present with N-terminal sequences SPPG and GNNP. The sequence of one peptide (designated ApN), SPPG, corresponds to the N-terminal sequence of band A and other bPRPs, while the sequence of the other peptide (designated ApC), GNNP, corresponds to that which might be expected after endoproteinase Glu-C cleavage of bPRP IB-6 or IB-8a. However, endoproteinase Glu-C

cleavage of IB-8a should result in three, rather than two, fragments (Table 2). The endoproteinase Glu-C digestion product (Ap) of band A did not act as a receptor for *C. albicans* adherence in overlay assays (Fig. 5).

MS of band Ap detected two species of masses 6189.07 ± 6.02 and 4261.14 ± 2.77 Da (mean $\pm$ SD of seven determinations). The mass of one of the species, 4261 Da, matched that expected for the C-terminal fragment of IB-6 (Table 2). The mass of the other species was 1085 Da smaller than that expected for the N-terminal endoproteinase Glu-C fragment of IB-6, and did not correspond to any of the cleavage products predicted for IB-6, IB-7, IB-8a, IB-8c or IB-9. The mass of the protein comprising band A could not be determined by MS.

DISCUSSION

Saliva is a complex mixture of ions, proteins, glycoproteins and polysaccharides of host and microbial origin (Gibson & Beeley, 1994). A number of salivary proteins and glycoproteins are adsorbed onto oral surfaces to form the acquired pellicle and in so doing provide many potential receptors for microbial adhesins (Bradway *et al.*, 1992). The objective of this study was to identify receptors in human parotid saliva for the adhesion of *C. albicans*.

Parotid saliva was studied because it can be obtained relatively free of microbial contaminants, and it was pooled in order to remove the effects of individual-to-individual saliva variations (Beeley *et al.*, 1991). The overlay assay has been used in a number of attempts to identify possible salivary receptors for microbial adhesion (Prakobphol *et al.*, 1987; Gillece-Castro *et al.*, 1991; Murray *et al.*, 1992; Hoffman & Haidaris, 1993, 1994). Binding of *Fusobacterium nucleatum* to the low-

Fig. 5. Binding of *C. albicans* ATCC 10261 cells to band A and the endoproteinase Glu-C digestion product, Ap. Proteins were separated by electrophoresis through a 16% polyacrylamide gel and electroblotted onto a nitrocellulose membrane. (a) Ponceau-S-stained nitrocellulose membrane. Lanes: 1, low-molecular-mass markers; 2, parotid saliva (15 μ g); 3, fractionated parotid saliva pool 3 (7.5 μ g); 4, band A; 5, band A plus 0.6 units endoproteinase Glu-C; 6, band A plus 0.3 units endoproteinase Glu-C. (b) Autoradiogram of the membrane in (a) probed with *C. albicans* ATCC 10261 cells. Lanes are the same as in (a). A, Band A; Ap, endoproteinase Glu-C digestion product of band A.

and high-molecular-mass PRGs present in human submandibular-sublingual saliva (Prakobphol *et al.*, 1987) has been demonstrated. Later work showed that *F. nucleatum* binds to the low- and high-molecular-mass PRG, via interactions with the unsubstituted lactosamine units, including the 6-antennae of the major oligosaccharide residues (Gillece-Castro *et al.*, 1991). Murray *et al.* (1992) studied the binding of 16 strains of streptococci to salivary glycoproteins present in parotid and submandibular-sublingual saliva. Three strains (*Streptococcus sanguis* 72-40 and 804, and *Streptococcus sobrinus* OMZ176) bound to single components, two to mucin MG2 and one to an unidentified protein, and five strains (*S. sanguis* 10556, *S. gordonii* 10558 and G9B, and *Streptococcus oralis* 10557 and 72-41) bound to multiple components, including MG2, α -amylase and PRGs.

Our previous study indicated that *C. albicans* bound to proteins in five separate pools of fractionated parotid saliva when adsorbed to hydroxylapatite beads (Cannon *et al.*, 1995). Therefore, it was not surprising that radiolabelled *C. albicans* cells bound to several electrophoretically separated parotid saliva proteins, immobilized on nitrocellulose in the overlay assay. We have also found that *C. albicans* cells bound to a similar set of proteins from unstimulated human whole saliva in overlay assays (unpublished observations).

Strain-specific variation was evident in the overlay profiles; however, a subset of four proteins acted as receptors for all *C. albicans* strains tested. The fact that *C. albicans* bound to several parotid saliva proteins may indicate the presence of either common receptors on different parotid salivary proteins, or multiple adhesins on the surface of *C. albicans*. We observed that strain-specific variation in the proportion of input cells binding to parotid saliva proteins in the overlay assay correlated with the level of adhesion observed in saliva-coated hydroxylapatite-bead assays. These variations may be related to either the avidity of adherence, or the number or accessibility of adhesins on the cell surface.

C. albicans cells bound to at least four proteins with sequence homology to nonglycosylated bPRPs (bands A–D). Protease digestion of band A with endoproteinase Glu-C resulted in a single band detected by Ponceau S or Coomassie blue staining after SDS-PAGE. This band contained two protein species with molecular masses of 4261 and 6189 Da, and two predominant N-terminal amino acid sequences. The presence in the digestion product of peptides with an N-terminal amino acid sequence and a molecular mass predicted for the C-terminal product of endoproteinase Glu-C digestion of bPRP IB-6 indicates that the protein in band A may be bPRP IB-6. The ApN fragment appears to be a degradation product of the N-terminal endoproteinase Glu-C fragment of IB-6 which is co-migrating with the smaller fragment. Discrepancies between the apparent molecular mass of band A and the calculated molecular mass are explained by the observation that PRPs migrate anomalously in SDS-PAGE (Anderson *et al.*, 1982). It appears that endoproteinase Glu-C cleavage of band A

results in loss of the *C. albicans* adhesion property. This may be due to proteolytic destruction of the adherence epitope, or conformational changes as a result of proteolysis.

The binding of *C. albicans* cells to immobilized bPRPs suggests that these salivary proteins may have a role in the adhesion of *Candida* cells in the oral cavity. *C. albicans* ATCC 10261 cells have been shown to adhere to both a carbohydrate moiety and proteins on the surface of immobilized *S. gordonii* cells (Holmes *et al.*, 1995b, 1996). Pretreatment of immobilized *S. gordonii* NCTC 7869 cells with whole saliva enhanced *C. albicans* ATCC 10261 cell binding (Holmes *et al.*, 1995a). Since *S. gordonii* NCTC 7869 and other primary colonizers of the enamel pellicle such as *S. parasanguis* and *S. sanguis* adsorb bPRPs (Heaney *et al.*, 1992; Newman *et al.*, 1993), it may be that these micro-organisms become coated with bPRPs and provide receptors for the binding of *C. albicans*. We are investigating whether *C. albicans* cells bind PRPs in the fluid phase. It has been shown that oral bacteria bind to adsorbed PRPs despite the presence of fluid-phase PRPs (Gibbons *et al.*, 1991), and this may also be the case with *C. albicans*. Preliminary assays have shown that preincubation of *C. albicans* cells with parotid saliva did not detectably reduce the levels of PRPs.

Johnsson *et al.* (1993) predict that IB-6 may be capable of interacting with hydroxylapatite and thus form part of the enamel pellicle. However, mucosal surfaces are the major sites of oral colonization by *C. albicans* (Arendorf & Walker, 1980). The enzyme transglutaminase has been shown to covalently link acidic PRPs to epithelial cells and may also be involved in the attachment of bPRPs to mucosal surfaces (Bradway & Levine, 1993). Therefore, it is possible that transglutaminase-catalysed linkage of bPRPs to epithelial cells may be another factor promoting the adherence of *C. albicans* cells. Alternatively, bPRPs adsorbed to bacteria already attached to epithelial cells may present adhesion receptors for *C. albicans* colonization.

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