Microbiology (2000), **146**, 41–48 Printed in Great Britain

Adhesion of *Candida albicans* to oral streptococci is promoted by selective adsorption of salivary proteins to the streptococcal cell surface

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Adhesion of *Candida albicans* to saliva-coated surfaces is an important early step in the colonization of the oral cavity. *C. albicans* cells also adhere to several species of oral streptococci including *Streptococcus gordonii*, *Streptococcus oralis* and *Streptococcus sanguinis* in what are believed to be multi-modal interactions. It is now demonstrated that incubation of streptococcal cells of these species with human parotid saliva further promotes the adhesion of *C. albicans* cells by up to 2·3-fold. Various species of streptococci were shown to adsorb different protein components of parotid saliva to their cell surfaces. The basic proline-rich proteins (bPRPs), to which *C. albicans* cells bind on nitrocellulose blot overlay, were strongly adsorbed to the surface of *S. gordonii* cells but not to *S. oralis* cells. Parotid saliva that was pre-adsorbed with *S. gordonii* cells and then applied to hydroxylapatite beads was <50% effective at supporting adhesion of *C. albicans* compared with control (non-adsorbed) saliva, demonstrating that bPRPs are major pellicle receptors. *C. albicans* cells did not adsorb bPRPs from fluid-phase parotid saliva. Following size-exclusion chromatography of parotid saliva samples, pooled fractions enriched in bPRPs promoted maximal adhesion of *C. albicans* to *S. gordonii* cells. The results demonstrate that *C. albicans* cells recognize only surface-bound forms of bPRPs and suggest that these proteins adsorbed to enamel or to streptococcal surfaces promote *C. albicans* adhesion and oral colonization.

Keywords: co-adhesion, *Candida albicans*, streptococci, proline-rich proteins

INTRODUCTION

Candida albicans is an opportunistic human pathogen that colonizes the oral cavities of a large proportion of the population without causing disease (Odds, 1988; Cannon *et al*., 1995a; Cannon & Chaffin, 1999). Under conditions of host immunosuppression, however, *C. albicans* can cause a number of mucosal infections, including oral candidiasis and *Candida* vulvovaginitis (Odds, 1988). Oral candidiasis is the collective term for a number of distinct clinical oral pathologies that include acute pseudomembranous candidiasis, erythematous candidiasis, chronic hyperplastic candidiasis, chronic atrophic candidiasis and angular cheilitis (Odds, 1988; Samaranayake, 1992). Chronic atrophic candidiasis (denture-induced stomatitis) is the most common clinical manifestation of oral candidiasis and occurs in up to 60% of denture wearers (Odds, 1988; Lynch & Tenn, 1994).

The oral cavity comprises diverse micro-environments containing a range of surfaces to which microbial cells can adhere, including the teeth, epithelial mucosa and the nascent surfaces created as micro-organisms bind to existing biofilms (Whittaker *et al*., 1996). All these surfaces are coated with salivary glycoproteins that are selectively bound (Douglas & Russell, 1984;

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Abbreviations: HA, hydroxylapatite; PRPs, proline-rich proteins; aPRPs, acidic proline-rich proteins; bPRPs, basic proline-rich proteins.

Scannapieco *et al*., 1989; Nikawa & Hamada, 1990; Edgerton *et al*., 1993; Newman *et al*., 1993; Rudney *et al*., 1995). Co-adhesion (the binding of planktonic microbial cells to surface-bound microbial cells) is an important mechanism in microbial cell growth and survival in the oral cavity, and may be modulated by salivary molecules.

C. albicans has been isolated from dentures (Arendorf & Walker, 1979; Wright *et al*., 1985), dental plaque (Hodson & Craig, 1972; Arendorf & Walker, 1980) and from sites of periodontal disease (Slots *et al*., 1988; Rams & Slots, 1991). Furthermore, adhesion of *C. albicans* to oral surfaces including buccal epithelial cells and acrylic (Verran & Motteram, 1987; Branting *et al*., 1989) has been shown to be influenced by oral bacteria. Therefore, there is good *in vivo* and *in vitro* evidence for *Candida* interactions with bacterial biofilms. Holmes *et al*. (1995b) demonstrated that *C. albicans* cells co-aggregate with, and co-adhere to, certain species of oral streptococci, and for some species, e.g. *Streptococcus gordonii*, salivary proteins enhance the interactions (Holmes *et al*., 1995a). Salivary-protein-enhanced co-adhesion of oral bacteria has been frequently described (see Jenkinson & Lamont, 1997) and the saliva-enhanced binding of *Streptococcus mutans* to *Actinomyces naeslundii*, *Streptococcus sanguinis* and *Streptococcus mitis* is believed to be a significant factor in the development of early plaque (Lamont & Rosan, 1990). However, few studies have identified the salivary components that enhance inter-microbial adhesion, or indeed that may inhibit co-adhesion (Stinson *et al*., 1992).

Several studies (Gibbons & Hay, 1988; Gibbons *et al*., 1991) have demonstrated that the acidic proline-rich proteins (aPRPs) can promote the adherence of *A. naeslundii* and *S. gordonii* cells to apatitic surfaces. These studies concluded that the acidic PRP-1 protein was cryptic in nature due to the fact that it did not bind to the cell surface of *A. naeslundii* LY7 cells, but rather is only recognized as a receptor for *A. naeslundii* LY7 and *S. gordonii* Blackburn cells when immobilized onto an apatitic surface (Gibbons & Hay, 1988; Gibbons *et al*., 1991). However, subsequent studies have shown that oral streptococci, when incubated with parotid saliva, adsorb salivary protein components including the aPRPs and some basic PRPs (bPRPs IB-1 to IB-9) onto their surfaces in a species- and strain-specific manner (Newman *et al*., 1993). Therefore, it is possible that some streptococci are able to recognize alternate forms of PRPs, either soluble or bound. This is analogous to the suggestion that streptococci are able to bind and differentiate between soluble and immobilized forms of salivary agglutinin (Jenkinson & Demuth, 1997) and allows bacterial cell adhesion to salivary pellicles despite the excess soluble forms of the receptor in saliva.

Since we have previously identified the bPRP IB-6 and three other PRPs as possible pellicle receptors for *C. albicans* ATCC 10261 adhesion (O'Sullivan *et al*., 1997), we investigated the hypothesis that selective adsorption of bPRPs by streptococci may enhance *C. albicans* adhesion within oral biofilms.

METHODS

Strains and growth media. The streptococcal strains used included *S. gordonii* NCTC 7869 (Channon), *S. gordonii* DL1 (Challis) (Pakula & Walczak, 1963), *S. sanguinis* ATCC 10556, *S. mitis* NCTC 10712, *Streptococcus oralis* 34 (from P. E. Kolenbrander, National Institutes of Health, Bethesda) and *S. mutans* NG8 (from A. S. Bleiweis, University of Florida, Gainesville). *C. albicans* ATCC 10261 was the yeast strain used in this study. Streptococcal cells were grown on TSBY agar (l^{-1}: 20 g trypticase soy broth, 5 g yeast nitrogen broth, 5 g glucose, 20 g agar) or in BHY (l^{-1}: 37 g brain heart infusion, 5 g yeast extract) medium at 37 °C in screw-capped tubes or bottles as stationary cultures (McNab *et al*., 1994). Late-exponential-phase cultures (OD_{600} 2, approx. 4×10^9 cells ml^{-1}) were centrifuged (6000 ***g***, 4 °C, 10 min) to pellet bacterial cells, which were then washed and prepared for assays as described below. *C. albicans* ATCC 10261 cells were grown in glucose salts biotin medium (GSB; Holmes & Shepherd, 1988) at 30 °C with vigorous shaking and collected from exponential-phase cultures by centrifugation (4000 ***g***, 10 min, 4 °C) (O'Sullivan *et al*., 1997).

Collection and fractionation of parotid saliva. Stimulated parotid saliva samples were collected from six donors using a modified Carlson–Crittenden device (Shannon *et al*., 1962) and stored on ice. The following proteinase inhibitors were added to the saliva samples (final concentrations): PMSF (1 mM); bisulphite (10 mM); benzamidine/HCl (2 mM); pepstatin A (2·9 μM); and aprotinin (0·3 μM). Samples were then pooled and clarified by centrifugation (12000 ***g***, 4 °C, 15 min). For fractionation, saliva (60 ml) was freeze-dried, suspended in water and applied to a Sephacryl S200 column (XK 26/100; Pharmacia). Fractions were collected and characterized as previously described (O'Sullivan *et al*., 1997).

Adhesion of *C. albicans* to saliva-coated hydroxylapatite (HA) beads. *C. albicans* cells were radioactively labelled with [^{35}S]methionine [New England Nuclear; $4{\cdot}3 \times 10^{13}$ Bq $mmol^{-1}$ (1175 Ci $mmol^{-1}$)] to a specific radioactivity of $0{\cdot}036 \pm 0{\cdot}017$ c.p.m. per cell as described previously (O'Sullivan *et al*., 1997). Labelled cells were washed and suspended in KCl buffer (2 mM KH_2PO_4, 2 mM K_2HPO_4, 5 mM KCl, 1 mM $CaCl_2$, pH 6·5) and numbers of *C. albicans* cells adhering to parotid-saliva-coated HA beads (20 mg; Macrosorb C, Phase Sep) were measured as described by Cannon *et al*. (1995b).

SDS-PAGE and electroblotting. SDS-PAGE was performed according to the method of Laemmli (1970) through 12·5 % (w/v) acrylamide gels. The apparent molecular mass of proteins was estimated using pre-stained protein markers (15·4–200 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; Amersham) for use in blot overlay assays. Transfer conditions were 100 V, 1·5 h, at 4 °C and membranes were stored in plastic wrap at 4 °C until use.

Blot overlay assay. The binding of *C. albicans* ATCC 10261 cells to electrophoretically separated parotid saliva proteins, immobilized on nitrocellulose membranes, was determined as previously described (O'Sullivan *et al*., 1997). Salivary proteins on blot membranes were first visualized by staining them with 0·2 % (w/v) Ponceau S in 1 % (v/v) acetic acid for 5 min, followed by four washes with 1 % (v/v) acetic acid. The membranes were destained with two washes (30 min, 20 °C) of PBS (l^{-1}: 8·5 g NaCl, 0·3 g KH_2PO_4, 0·6 g Na_2HPO_4, pH

7·5) and blocked with 5% (w/v) BSA in KCl buffer (2 h, 20 °C). Membranes were incubated with radioactively labelled *C. albicans* cells [30 ml, $1{\cdot}1 \times 10^7$ cells (ml KCl buffer)$^{-1}$] for 18 h at 4 °C with reciprocal shaking (70 min^{-1}), washed four times in PBS containing 0·1% (v/v) Tween 20 to remove non-specifically bound *C. albicans* cells and air-dried prior to exposure to X-ray film.

Fluid-phase adsorption assay. Proteins were adsorbed from parotid saliva by microbial cells following a modification of the method described by Newman *et al.* (1993). Streptococcal or candidal cells were harvested from cultures by centrifugation, washed with KCl buffer as described above, and suspended in KCl buffer at a concentration of 8×10^7 streptococcal cells ml^{-1} or $4{\cdot}4 \times 10^7$ *C. albicans* cells ml^{-1}. Bacterial or yeast cell suspensions (0·3 ml) were added to parotid saliva diluted to 0·5 mg protein ml^{-1} with KCl buffer (0·6 ml) and incubated with end-over-end rotation (9 r.p.m.) at 20 °C for 1 h. Cells were sedimented by centrifugation (2900 ***g***, 4 °C, 10 min) and the supernatant was collected and analysed by SDS-PAGE or retained as pre-adsorbed parotid saliva samples for use in blot overlay and adhesion assays.

Coadhesion assay. *C. albicans*–streptococcal co-adhesion assays were performed essentially as described by Holmes *et al.* (1995a, 1996). Streptococcal or candidal cells were washed and suspended at a concentration of 4×10^8 cells ml^{-1} or $4{\cdot}4 \times 10^7$ cells ml^{-1}, respectively, in TNMC (1 mM Tris/HCl, pH 8·0, containing 0·15 M NaCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$). Bacterial or yeast cell suspensions (50 µl) were dispensed into 96-well microtitre plates (Scintistrip, Wallac Oy), centrifuged (800 ***g***, 5 min, 20 °C) to immobilize cells, and the supernatant was discarded. The wells were washed twice [150 µl, 0·05%, v/v, Tween 20 in TNMC buffer (Tween-TNMC)] and incubated with Tween-TNMC (150 µl, 16 h, 4 °C). The liquid was aspirated from the wells and 50–150 µl portions of saliva samples (diluted in TNMC buffer) were added and incubated at 20 °C for 1 h. The wells were washed twice (150 µl Tween-TNMC) and then incubated with radiolabelled *C. albicans* cells [0·1 ml, $2{\cdot}5 \times 10^6$ cells (ml TNMC buffer)$^{-1}$, 20 °C, 2 h, with shaking (50 r.p.m.)]. Liquid was aspirated from the wells, which were washed four times (150 µl Tween-TNMC, 10 min) before allowing them to dry (37 °C, 1 h). The plastic well strips were snap-separated and bound radioactivity was counted using a 1450 Microbeta Plus liquid scintillation counter (LKB, Wallac Oy).

RESULTS

Effect of parotid saliva on *C. albicans* co-adhesion

The co-adhesion of *C. albicans* ATCC 10261 cells to immobilized streptococcal cells was measured in a microtitre well assay. Substantial proportions of input yeast cells (24–49%) adhered to *S. gordonii*, *S. oralis* or *S. sanguinis* cells, while much fewer cells bound to *S. mutans* cells (Fig. 1). *C. albicans* demonstrated low-level binding to immobilized homologous candidal cells. *S. oralis* 34 and *S. sanguinis* ATCC 10556 supported greater numbers of *C. albicans* bound (up to 49% of input cells) compared with *S. gordonii* DL1 cells, confirming previous results (Holmes *et al.*, 1995a). Pre-incubation of streptococcal cells with parotid salivary proteins (equivalent to approximately 2 pg protein per microbial cell) increased markedly the binding of *C. albicans* to *S. gordonii* (2·3-fold) and also significantly

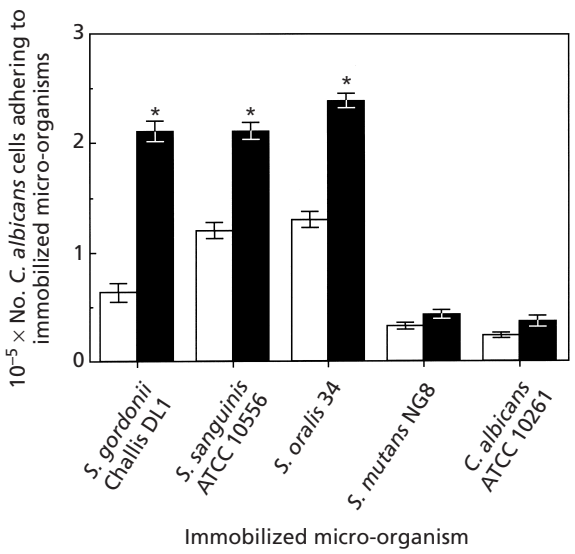

Fig. 1. Stimulation of *C. albicans* ATCC 10261 cell adhesion to streptococci by parotid saliva. Streptococcal or *C. albicans* cells (2×10^7 or $2{\cdot}2 \times 10^6$, respectively) immobilized to microtitre plate wells were incubated with diluted parotid saliva (black bars; equivalent to approx. 2 pg salivary protein per cell) at 20 °C for 1 h, or with buffer alone (white bars), prior to addition of radiolabelled *C. albicans* cells ($2{\cdot}6 \times 10^5$ cells per well). Representative results, which are the means (±SD) of triplicates performed on the same batch of cells, are shown. Background binding to buffer-treated wells was $2{\cdot}6 \pm 2{\cdot}08 \times 10^3$ *C. albicans* cells. *Significant increase in adherence ($P < 0{\cdot}005$, Student's *t*-test).

($P < 0{\cdot}005$) increased binding of *C. albicans* cells to *S. sanguinis* ATCC 10556 and *S. oralis* 34 cells (Fig. 1). Saliva did not, however, significantly affect the binding of yeast cells to *S. mutans* or to homologous *C. albicans* cells (Fig. 1). Preliminary, as well as subsequent, experiments showed that this salivary stimulation of the numbers of *Candida* binding to streptococci was dose-dependent (saturating at approximately 1·4 pg salivary protein per *S. gordonii* cell), and in all instances was maximal at the concentration of salivary proteins (equivalent to approximately 2 pg per bacterial cell) used in the experiment shown in Fig. 1. These results indicated that streptococcal cells selectively adsorbed salivary protein receptors for *C. albicans* adhesion and therefore we proceeded to determine the nature of these receptors.

Adsorption of salivary proteins by streptococcal cells

To identify the protein components depleted from saliva by streptococci, adsorbed saliva samples were analysed by SDS-PAGE and the candidal adhesion receptors remaining were determined by blot overlay. Incubation of saliva with *S. gordonii* NCTC 7869 (Fig. 2) or DL1 (data not shown) cells caused a depletion in protein bands of apparent molecular masses 72, 42, 32, 28, 24, 16·5 and 15 kDa, compared with saliva samples incubated under identical conditions without bacteria added.

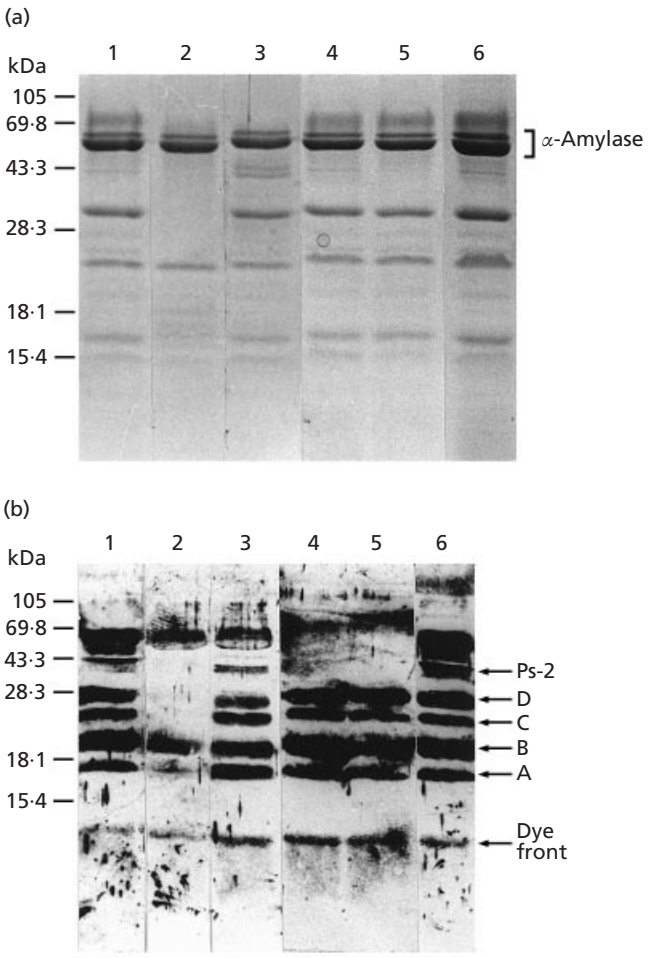

Fig. 2. Selective adsorption of salivary proteins by oral streptococci. Clarified parotid saliva was incubated with streptococcal or *C. albicans* ATCC 10261 cells at 20 °C for 1 h. Microbial cells were sedimented by centrifugation and supernatants (20 μl per lane) were subjected to SDS-PAGE. Gels were either stained with Coomassie blue R250 or electroblotted onto nitrocellulose membranes which were then incubated with radiolabelled *C. albicans* cells ($1{\cdot}03 \times 10^7$ cells ml^{-1}). (a) Coomassie-stained gel; (b) autoradiogram of corresponding blot overlay. Micro-organisms used to adsorb salivary proteins were: *C. albicans* ATCC 10261, lane 1; *S. gordonii* NCTC 7869, lane 2; *S. oralis* 34, lane 3; *S. mitis* NCTC 10712, lane 4; *S. sanguinis* ATCC 10556, lane 5; KCl buffer control, lane 6. A–D are bands previously identified as receptors for *C. albicans* adherence (O'Sullivan *et al.*, 1997) and Ps-2 is the basic PRP-2. The experiment was carried out three times and representative results are shown.

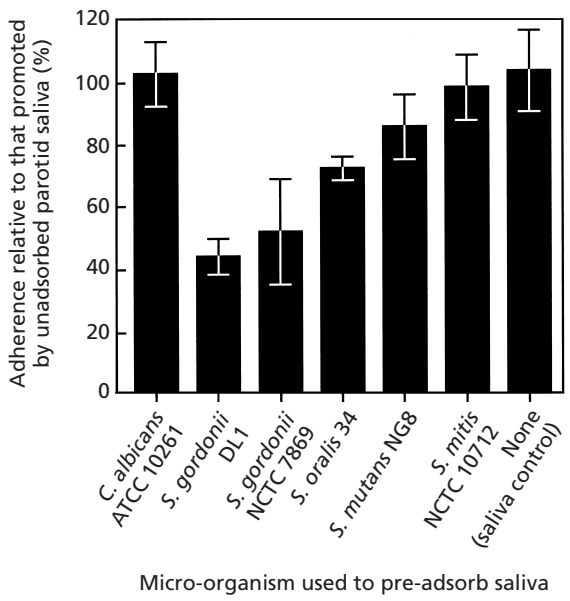

Fig. 3. Effect of pre-adsorbing saliva with oral micro-organisms on subsequent ability to support *C. albicans* adhesion to HA beads. Pre-adsorbed saliva samples were used to coat HA beads and the numbers of *C. albicans* ATCC 10261 cells binding (input $2{\cdot}7 \pm 0{\cdot}14 \times 10^6$) were measured. Results are expressed as a percentage of the binding promoted by the unadsorbed parotid saliva sample ($9{\cdot}72 \pm 1{\cdot}35 \times 10^5$ cells). Results are the means (±SD) of triplicates performed on at least one batch of cells.

S. oralis 34 or *S. sanguinis* ATCC 10556 cells also removed the 72 or 42 kDa protein bands, respectively (Fig. 2a, lanes 3 and 5). By contrast, incubation of saliva with cells of *S. mitis* NCTC 10712, *S. mutans* NG8 or *C. albicans* ATCC 10261 had no significant effects on the protein profiles (Fig. 2). *S. gordonii* binds α-amylase with high affinity from fluid-phase parotid saliva (Rogers *et al.*, 1998; Scannapieco *et al.*, 1989), but under the assay conditions described the putative α-amylase was not completely removed by any strain tested (see Fig. 2). This was confirmed by demonstrating the presence of α-amylase activity, using starch agar plates (Douglas *et al.*, 1990), in saliva samples that had been pre-incubated with streptococcal cells (data not shown).

We have previously identified four salivary bPRPs (denoted band A, 17 kDa; band B, 20 kDa; band C, 24 kDa; and band D, 27 kDa) that are bound by *C. albicans* cells in blot overlay assays (O'Sullivan *et al.*, 1997). In addition, *C. albicans* cells also bind to the bPRP Ps-2 (43 kDa). Following incubation of parotid saliva samples with *S. gordonii* cells, no binding of *C. albicans* cells to band C or band D was observed (Fig. 2b) and the 43 kDa (Ps-2) receptor for *C. albicans* present in untreated saliva (Fig. 2) was also removed. Binding of *C. albicans* cells to band A (17 kDa, bPRP IB-6; O'Sullivan *et al.*, 1997) and band B (20 kDa) was also reduced (Fig. 2b). Pre-incubation of parotid saliva with *S. mitis* NCTC 10712 or *S. sanguinis* ATCC 10556 also removed the 43 kDa *C. albicans* ATCC 10261 adhesion receptor Ps-2 (Fig. 2b, lanes 4 and 5). Incubation of parotid saliva with *S. oralis*, *C. albicans* ATCC 10261 (Fig. 2b) or with *S. mutans* (data not shown) did not alter the binding profiles of *C. albicans* in blot overlay assays. These results demonstrated that the various streptococcal species adsorbed different salivary proteins to their cell surfaces, and suggested that the adsorption of bPRPs by *S. gordonii* could account for the *C. albicans*–*S. gordonii* co-adhesion-promoting ac-

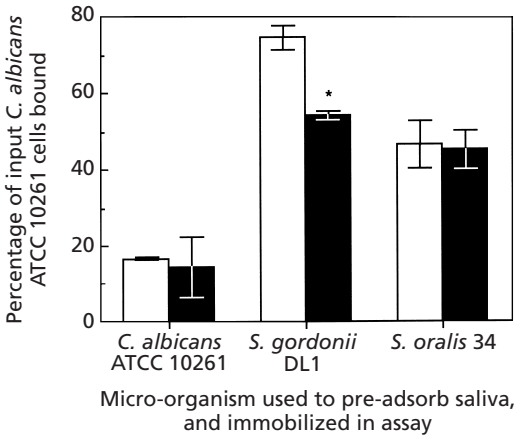

Fig. 4. Effect of pre-adsorbing parotid saliva on the ability to support *C. albicans* cell adhesion to immobilized micro-organisms. Saliva was pre-adsorbed with streptococcal or *C. albicans* cells and then used in microtitre assays to measure adhesion of *C. albicans* cells to fresh, immobilized, micro-organisms. Numbers are the percentage of the input *C. albicans* ATCC 10261 cells ($2{\cdot}6 \pm 10^5$ per well) bound, and are the means of quadruplicates. White bars, unadsorbed saliva; black bars, pre-adsorbed saliva. *Significant decrease in adherence ($P < 0{\cdot}05$, Student's *t*-test).

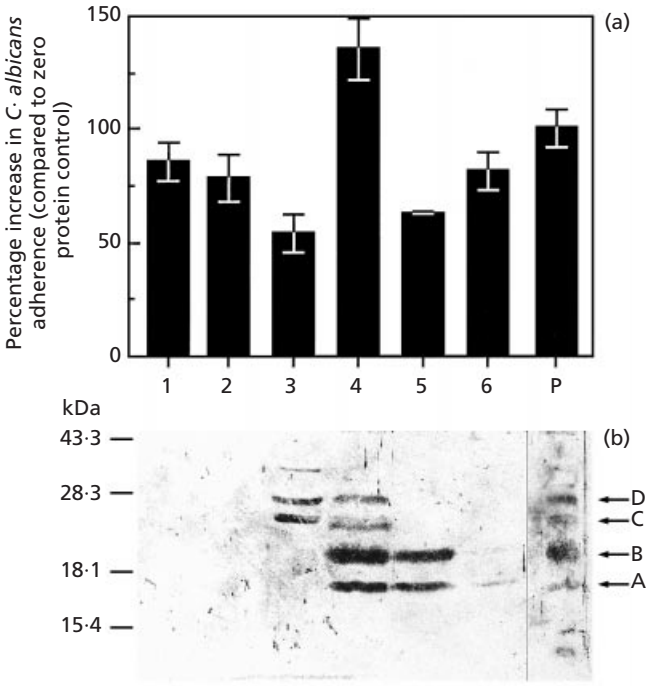

Fig. 5. Saliva proteins that promote adhesion of *C. albicans* to *S. gordonii* cells. Pooled parotid saliva was fractionated by Sephacryl S200 column chromatography. Pooled fractions were utilized in microtitre well assays, and subjected to blot overlay analysis. (a) Percentage increase in numbers of *C. albicans* ATCC 10261 cells adhering to immobilized *S. gordonii* DL1 coated with salivary proteins from pools 1 through 6 or by parotid saliva (P) (approx. 2 pg salivary proteins applied per cell), compared with control (no saliva; $17{\cdot}5 \pm 0{\cdot}77$% input cells adhered). Results are the means (±SD) of triplicates performed on the same batch of cells. (b) *C. albicans* ATCC 10261 blot overlay of parotid saliva pool samples in (a). Parotid saliva pools 1–6 (20 µl per lane) and parotid saliva (P; 8 µg) were separated by SDS-PAGE, electroblotted onto nitrocellulose membranes and incubated with radiolabelled *C. albicans* cells ($1{\cdot}1 \times 10^7$ cells ml^{-1}).

tivity of parotid saliva. Conversely, *S. oralis* 34 did not appear to adsorb bPRPs and thus salivary stimulation of *C. albicans*–*S. oralis* co-adhesion must occur independently of bPRPs.

Proteins bound by streptococci also support adhesion of *C. albicans* to experimental pellicle

To determine if the same salivary components that promote binding of *C. albicans* to streptococci also promote adherence of *Candida* cells to saliva-treated HA beads, parotid saliva samples were pre-treated with streptococcal cells and then used to coat HA beads. There was a >50% reduction in the numbers of *C. albicans* cells binding to HA beads coated with saliva that had been pre-incubated with *S. gordonii* cells, compared with untreated saliva (Fig. 3). A reduction (28%) was also noted in numbers of yeast cells adhering to *S. oralis*-treated saliva-coated beads, but there was no significant effect on the numbers binding to HA beads coated with saliva that had been pre-incubated with *S. mitis*, *S. mutans* or *C. albicans* (Fig. 3).

As expected, parotid saliva that had been pre-incubated with *S. gordonii* cells was significantly ($P < 0{\cdot}05$, *t*-test) reduced in its ability to promote *C. albicans*–*S. gordonii* DL1 co-adherence (Fig. 4). By contrast, parotid saliva pre-incubated with *S. oralis* 34 cells, or with *C. albicans* ATCC 10261, was not significantly reduced in stimulation of co-adhesion (Fig. 4). These data confirm that the major enamel pellicle receptors for *C. albicans* are also subject to high-affinity adsorption to the cell surface of *S. gordonii*.

Promotion of *C. albicans*–streptococcal co-adhesion by parotid salivary protein fractions

To confirm that the bPRPs within parotid saliva were capable of directly promoting *C. albicans* adhesion to *S. gordonii*, adhesion-promoting activities in salivary protein fractions obtained following size-exclusion column chromatography were measured (Fig. 5). Pools designated 1 through 6 contained salivary proteins as previously characterized (O'Sullivan *et al.*, 1997), with pool 4 being enriched in bPRP bands A–D (Fig. 5b). The greatest stimulation of *C. albicans* adhesion to *S. gordonii* was associated with pool 4, while protein pools 3 or 5, which also contained lesser amounts of bPRPs, also promoted adhesion. Pool 1, which contains protein aggregates excluded from the column, also stimulated a high level of *C. albicans* adhesion to *S. gordonii*. The ability of pools 1, 2 and 6 to promote *C. albicans*–*S. gordonii* co-adhesion indicates that other proteins, or protein complexes, within saliva also act as receptors for

C. albicans adherence. However, the magnitude of the adherence stimulation obtained with pool 4, containing all four bPRPs, indicates that the bPRPs are of greatest significance.

DISCUSSION

Colonization of the oral cavity by *C. albicans* is a crucial step in the sequence of events leading to an infection. Salivary proteins and glycoproteins can act as receptors for binding of *C. albicans* cells to enamel surfaces (Cannon *et al*., 1995b) and to denture acrylic surfaces (Vasilas *et al*., 1992; Edgerton *et al*., 1993; Nikawa *et al*., 1993) and can modulate the binding of yeast cells to buccal epithelial cells (Kimura & Pearsall, 1978; Samaranayake & MacFarlane, 1982). In addition, *C. albicans* binding to oral viridans streptococci (Jenkinson *et al*., 1990; Holmes *et al*., 1995a, b, 1996) and to *Actinomyces* (Grimaudo *et al*., 1996) may be enhanced by salivary components (Holmes *et al*., 1995a; Grimaudo *et al*., 1996). We have now determined that the basic PRPs present in human parotid salivary secretions are not only active in providing receptors for adhesion of *C. albicans* to enamel pellicles, but also that these salivary components are adsorbed by streptococci and act to promote adhesion of *C. albicans* ATCC 10261 to bacterial cells.

Salivary proteins and glycoproteins are present in multiply active conformations within the human oral cavity. In the fluid phase, salivary components may be present as discrete molecules or more usually as macromolecular complexes often involving mucins (Iontcheva *et al*., 1997). A number of these components are selectively adsorbed onto enamel, and the aPRPs and statherin change conformation upon binding to enamel (Moreno *et al*., 1984) thus unmasking receptors for adhesion of streptococci and *Actinomyces* species (Gibbons & Hay, 1988; Gibbons *et al*., 1990, 1991). This ability of oral micro-organisms to bind immobilized salivary proteins is of considerable ecological significance. Adhesins that have evolved to bind immobilized conformations of salivary components provide a mechanism for attachment of microbial cells to oral surfaces despite the presence of excess soluble forms of the receptors within saliva. The bPRPs appear to comprise another group of salivary proteins that act as receptors for microbial cell adhesion when immobilized. Interestingly, the streptococci, in particular *S. gordonii*, are able to adsorb these proteins to their surfaces and the bound forms then act as receptors for *C. albicans* adhesion. Conversely, *C. albicans* cells are unable to adsorb bPRPs from saliva, although small amounts of other macromolecules are adsorbed by yeast cells (Edgerton *et al*., 1993). Thus *C. albicans* demonstrates a unique adhesive mechanism whereby adhesion may occur to surface-bound forms of bPRPs despite the presence of excess fluid-phase bPRPs in saliva.

Streptococci are early colonizers of salivary pellicles and their ability to bind salivary proteins and glycoproteins is important in plaque development. Of the streptococcal species tested, *S. gordonii* cells bound salivary proteins to the greatest extent, but the components of saliva bound by the streptococcal species varied quite considerably. The changes to the salivary protein profiles observed following incubation with streptococcal cells were highly reproducible, and evidence suggested that the alterations resulted from adsorption of the proteins to the bacterial cell surface rather than for other reasons such as proteolysis. Even in the presence of a cocktail of protease inhibitors, bPRP bands A, C and D were adsorbed by *S. gordonii* within 15 min determined by the overlay assay (data not shown). Previously, *S. gordonii* cells have been reported to bind to PRPs IB-1 to IB-9 and Ps-1 in blot overlay assays (Newman *et al*., 1996). The adsorption of bPRPs by *S. gordonii* occurs via a high-affinity mechanism since maximal adsorption of bPRPs from the fluid phase occurred under conditions that did not result in the complete removal of α-amylase, which is a high-affinity binding reaction (Scannapieco *et al*., 1989). Pool 4 promotes high binding of *C. albicans* to immobilized *S. gordonii* cells and it is possible that all four bPRPs are recognized, when presented on the surface of the bacterial cells, with a concomitant increase in binding activity. As a result of adsorption of bPRPs by streptococci, the PRP-depleted saliva was much less efficient at supporting *C. albicans* adhesion to HA beads, confirming that similar forms of the adhesion receptors are presented by enamel and by streptococci.

S. gordonii is found at multiple sites within the human oral cavity and is associated with numerous intra- and inter-generic bacterial co-aggregations which are postulated to be involved in the formation of oral biofilms (Kolenbrander & London, 1993; Holmes *et al*., 1996). Therefore, adhesion of *C. albicans* cells to salivary components adsorbed to *S. gordonii* cells would potentially increase the number of available sites for *C. albicans* adhesion and colonization. In support of this suggestion, it has been observed that *C. albicans* cells are more usually found associated with plaque-coated areas of enamel (Arendorf & Walker, 1980). It could be envisaged that presentation of bPRPs on the surface of streptococci might also tend to co-aggregate *C. albicans* and promote clearance from the oral cavity. However, streptococcal cells also bind to aPRP 1-coated enamel (Gibbons *et al*., 1991). Thus direct co-aggregation of *C. albicans* with streptococci, or co-aggregation via bPRP, followed by binding of streptococci to an oral surface could also provide a means by which *C. albicans* colonization is promoted.

Currently, the molecular nature of the receptor that binds bPRPs to the streptococcal cell surface is not known. However, two protein families on the streptococcal cell surface are known to bind to other salivary proteins. The antigen I/II proteins (Jenkinson & Demuth, 1997) bind salivary agglutinin glycoprotein while AbpA (Rogers *et al*., 1998) binds α-amylase. The antigen I/II proteins SspA and SspB are also involved in the direct binding of *S. gordonii* to *C. albicans* cells (Holmes *et al*., 1996). We have observed that mutants

deficient in the production of SspA and SspB are reduced, by approximately 20 %, in their ability to adsorb bPRPs (unpublished observations). However, since adsorption of bPRPs by wild-type cells appears to be a rapid and high-affinity process, simply reduced adsorption by the mutants implies that the SspA and SspB proteins are not the major bPRP adhesins. Therefore, it seems likely that the binding of bPRPs to the streptococcal cell surface may occur through a multi-modal mechanism.

In summary, *C. albicans* cells do not appear to bind salivary bPRPs in the fluid phase. However, adsorption of these proteins to a surface such as enamel, *Streptococcus*, or to nitrocellulose *in vitro* exposes receptors that are then recognized by *C. albicans* cells. This ability to adhere to only surface-bound peptides would enable *C. albicans* cells to adhere to multiple oral cavity surfaces in the presence of fluid-phase saliva and thus enhance its colonization potential.

ACKNOWLEDGEMENTS

We thank A. S. Bleiweis, P. S. Handley and P. E. Kolenbrander for providing strains. J. M. O'Sullivan was in receipt of a Targeted Otago Research Grant for part of this work.

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Received 2 August 1999; revised 28 September 1999; accepted 14 October 1999.