Glucan-binding Proteins of *Streptococcus mutans* Serotype *c*

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Three glucan-binding proteins have been isolated from the extracellular fluid of cultures of *Streptococcus mutans* serotype *c*. These proteins were adsorbed to glucans containing 1,3- α or 1,6- α bonds and linked to various chromatographic supports: they were eluted from columns by a dextran solution. Glucosyltransferase activity was associated with two of the glucan-binding proteins.

INTRODUCTION

A prime determinant of the ability of *Streptococcus mutans* to form dental plaque is the capacity of the organism to synthesize high molecular weight glucans from sucrose (Gibbons & van Houte, 1975). The glucans formed are dextran, which consists of 1,6- α -linked glucose units, and mutan, a branched polymer with both 1,3- α and 1,6- α linkages (Baird *et al.*, 1973). The enzymes synthesizing these glucans are referred to collectively as glucosyltransferases. Mutan appears to be particularly important in the adherence process: the greater the amount of mutan produced, the more adherent the organism is (Michalek *et al.*, 1975). Conversely, mutants deficient in the ability to make mutan fail to form plaque *in vitro* (Freedman & Tanzer, 1974; Johnson *et al.*, 1977; de Stoppelaar *et al.*, 1971). Dextran also seems to contribute to plaque formation, perhaps by mediating cell-to-cell agglutination (Gibbons & Fitzgerald, 1969), and other sucrose-independent processes may be involved in the initial attachment of bacteria to tooth surfaces (Clark & Gibbons, 1977).

Reports from several laboratories have established the involvement in glucan binding of some protease-sensitive structures on the cell surface (Kelstrup & Funder-Nielsen, 1974; Liljemarm & Schauer, 1975; McCabe & Smith, 1975; Spinell & Gibbons, 1974); these receptor proteins might be specific glucan-binding proteins or could possibly be the glucosyltransferases themselves [although the active site of the enzyme is not involved in dextran binding (McCabe *et al.*, 1976)].

Recently a dextran-binding protein which is possibly involved in adherence was isolated from *S. mutans* strain 6715, serotype *g* (McCabe *et al.*, 1977; McCabe & Smith, 1977). This paper describes the identification of several proteins which are capable of binding to a variety of glucans in a serotype *c* strain of *S. mutans*.

METHODS

Organisms and growth conditions. *Streptococcus mutans* strain 3209, serotype *c*, from Dr J. A. Cole, University of Birmingham, is a recent human isolate. It is more readily agglutinated by dextran, and more efficient at producing mutan and adhering to surfaces, than our laboratory stock strains of serotype *c*. The other *S. mutans* strains examined were Ingbritt, FW293, NCTC 10449, C67-1, GS-5 and OMZ-70 (all serotype *c*) and K1 (serotype *g*).

Bacteria were grown for 16 h at 37 °C in the chemically defined medium described by Janda & Kuramitsu

(1976), supplemented with cysteine and sodium carbonate (Terleckyj & Shockman, 1975). Bacteria were harvested by centrifuging at 10000 *g* and the supernatant was clarified by filtration through glass-fibre (Whatman GF/D).

Bacteria suspended in 0.05 M-KH₂PO₄/K₂HPO₄ buffer (pH 6.5) at 4 °C were disintegrated with an MSE 150 W ultrasonic disintegrator at maximum output for 20 min. Unbroken bacteria and wall fragments were removed by centrifugation. Sodium azide was added to 0.1 % (w/v) to inhibit bacterial contamination before the filtrate or sonicated bacterial suspension was passed through the affinity chromatography columns.

Affinity columns. Columns of 20 to 50 ml were used with the following packings. (i) Sephadex G-25 coarse (Pharmacia); Sephadex consists of dextran cross-linked by epichlorhydrin. (ii) Dextran T10-Sepharese, prepared by coupling dextran (average molecular weight 10000; Pharmacia) to epoxy-activated Sepharese (Pharmacia) following the procedure outlined by the manufacturers. (iii) Dextran 2000-Sepharese, consisting of dextran (molecular weight 2000000; Pharmacia) coupled to epoxy-activated Sepharese. (iv) Blue Dextran-Sepharese, made by coupling Blue Dextran 2000 (Pharmacia) to cyanogen bromide-activated Sepharese (Pharmacia) by the procedure of Ryan & Vestling (1974). This column was used in conjunction with one containing Cibacron Blue F3GA coupled to Sepharese (Heyns & de Moor, 1974) in order to remove proteins with affinity for the dye. Cibacron Blue F3GA, the dye present in Blue Dextran 2000, was a gift of the CIBA-Geigy Co. (v) Mutan-P2. Mutan was prepared by incubating the culture filtrate of *S. mutans* with 5 % (w/v) sucrose at 37 °C for 48 h. The insoluble mutan was collected by centrifuging at 10000 *g*, washed twice with water, once with 6 M-guanidine hydrochloride, then three times more with water. The column of mutan plus BioGel P2 (Bio-Rad) was prepared as described by McCabe & Smith (1977), using 50 mg mutan with 20 ml of settled gel. (vi) Pseudonigeran-P2. Pseudonigeran (linear 1,3- α -linked glucan) was kindly provided by Dr G. J. Walker, Institute of Dental Research, Sydney, Australia. The affinity column was prepared as described for mutan (v).

Chromatography procedure. Unconcentrated culture filtrate or sonicated bacterial suspension was passed down an affinity column at a flow rate of 40 ml h⁻¹. The column was washed with several volumes of 0.05 M-KH₂PO₄/K₂HPO₄ buffer (pH 6.5) and eluted with 0.1 % (w/v) Dextran T10, 0.1 % (w/v) sodium dodecyl sulphate (SDS), 6 M-guanidine hydrochloride or 3 M-sodium thiocyanate in phosphate buffer. Eluates were concentrated by extensive dialysis against distilled water followed by lyophilization. Proteins were precipitated from the culture filtrate at 60 % saturation with ammonium sulphate and were collected after 16 h at 4 °C by centrifuging at 10000 *g*. The precipitated proteins were then dialysed against buffer.

Polyacrylamide gel electrophoresis. Electrophoresis in the presence of SDS using the buffer system of Laemmli (1970) with 8 % (w/v) acrylamide in the separating gel was done in an apparatus built to the design of Studier (1973). For SDS-polyacrylamide gradient gel electrophoresis, the same buffer system and apparatus were used; the 6 to 20 % acrylamide gradient was formed with a simple two-chamber mixing device.

Gels were fixed in 12 % (w/v) trichloroacetic acid and stained in 0.2 % (w/v) Coomassie Brilliant Blue in acetic acid/ethanol/water (10:45:45, by vol.). Destaining was in a similar solvent mixture using methanol instead of ethanol. Glucosyltransferase activity on gels was detected by incubation in 0.05 M-sodium acetate buffer (pH 5.5) containing 5 % sucrose and 1 % (w/v) Lubrol PX (Sigma) as described elsewhere (Russell, 1979).

RESULTS AND DISCUSSION

The protein composition of culture filtrates was complex, with at least 30 discrete bands being resolved under the electrophoretic conditions used (Fig. 1*b*). Nearly all of these proteins passed straight through the Sephadex G-25 affinity column but a few were retained and could subsequently be eluted by either low molecular weight dextran, SDS, or the chaotropic agents sodium thiocyanate or guanidine hydrochloride (Fig. 1*c*). All of the eluting agents released the same proteins from the affinity column, though after dextran elution further protein could be eluted by the other, more powerful, agents. The same proteins were retained by the column when the applied sample was a sonicated bacterial suspension.

Glucosyltransferases of serotype *c* strains have a very high molecular weight and migrate during SDS-gel electrophoresis to a position corresponding to that of the top pair of proteins eluted from the affinity column (Russell, 1979). Glucosyltransferase activity was detected in these two bands when a gel was incubated with sucrose. The third band had no detectable activity. Comparison of the mobility of the proteins from the affinity

Fig. 1. SDS-polyacrylamide gel electrophoresis of (a) molecular weight standards (from top): β -galactosidase (116000), phosphorylase (94000), bovine serum albumin (68000), ovalbumin (43000), chymotrypsinogen A (25700); (b) concentrated culture filtrate; (c) proteins bound by Sephadex G-25; (d) proteins bound by dextran T10-Sepharose; (e) proteins bound by mutan-P2; (f) proteins eluted from nascent mutan.

column with those of the standards shown in Fig. 1(a), using the revised molecular weight of 116000 for β -galactosidase (Fowler & Zabin, 1978) and including myosin (molecular weight 200000), allowed estimation of the molecular weights of the glucosyltransferases as 140000 and 132000, while the lower band corresponded to a molecular weight of 74000. This latter protein was of a similar size to the fructosyltransferases described recently (Russell, 1979) but no fructosyltransferase activity was present in affinity column eluates. Electrophoresis experiments in which samples containing fructosyltransferase were compared with the 74000 molecular weight protein confirmed that they were distinct.

The process by which dextran is cross-linked to prepare Sephadex gel beads might alter the native dextran configuration, so affinity columns in which dextran had been immobilized by other means were investigated. The same set of proteins as shown in Fig. 1(c) bound to columns of Sepharose containing dextran of molecular weight 10000 or 2000000 (Fig. 1d) and also to a column to which Blue Dextran had been coupled. When the mutan-P2 column was studied, again the same proteins were recovered (Fig. 1e). The faint bands visible in Fig. 1(e) were not obtained reproducibly. Mutan synthesized by the glucosyltransferases of strains 3209, Ingbritt or K1 were equally effective.

Recently, Smith *et al.* (1978) have described a method for preparing glucosyltransferase by releasing bound enzyme from its product (mutan) with guanidine hydrochloride. Figure 1(f) shows the proteins released by guanidine hydrochloride during the preparation of mutan synthesized by extracellular enzymes of strain 3209. Clearly, together with glucosyltransferase, this procedure also yielded the other dextran-binding protein. It is possible that a similar protein was present in the material used by Smith *et al.* (1978) to immunize hamsters against caries.

The results showing that the same proteins could bind to dextran or mutan columns,

together with the evidence that soluble dextran could elute the bound proteins from either type of column, suggested that the proteins bound to regions of 1,6- α -linked rather than to 1,3- α -linked glucan. Alternatively, they might be able to bind to any glucose polymer. To investigate these possibilities, a culture filtrate was passed down a column containing pseudonigeran. The column was eluted with guanidine hydrochloride and the protein composition of the eluate was examined by SDS-gel electrophoresis. The same three bands as those recovered from the dextran and mutan columns were found. No proteins bound to a column containing BioGel P2 alone. It appears, therefore, that all of the proteins recognize and bind to 1,6- α -linked glucans characteristic of dextran and also to the 1,3- α -linked regions present in mutan and pseudonigeran. No proteins with a unique binding specificity for 1,3- α -linked regions were observed.

Six other laboratory stock strains of serotype *c* were found to contain the same glucan-binding proteins as strain 3209, with only minor differences in the relative proportions of each protein being observed.

The role, if any, of the dextran-binding proteins in either dextran-mediated agglutination or cell-to-surface adherence is as yet unknown, though the failure to detect a specific mutan-binding protein suggests that the same receptor protein(s) may be involved in both processes. If any of these binding proteins are essential for agglutination and/or adherence, it may be that 'non-sticky' mutants will be shown to lack one or more of the glucan-binding proteins.

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