PURIFICATION OF DEXTRAN-BINDING PROTEIN FROM CARIOGENIC STREPTOCOCCUS MUTANS

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Summary

An extracellular protein produced by Streptococcus mutans was purified to electrophoretic homogeneity by affinity chromatography on Sephadex G50 followed by gel filtration. The protein is devoid of both dextransucrase and dextranase activity but binds dextran and therefore probably is implicated in the adherence of S. mutans cells to the host tooth surface. The presence of the dextran-binding protein may be a determinant of the pathogenicity of such cariogenic micro-organisms.

Introduction

Moscona proposed that macromolecules present on the cell surface mediate cell recognition and intercellular adherence (1). Recent observations indicate that cell-surface carbohydrate-binding proteins (resembling lectins) and complementary heterogeneous oligosaccharides present in the glycocalyces of adjacent cells mediate developmentally-regulated cell adherence phenomena in several organisms. Two developmentally regulated, galactose-binding lectins have been purified from the aggregating amoebae of cellular slime molds: discoidin, from Dictyostelium discoideum and pallidin, from Polysphondylium pallidum (2,3). Both have been strongly implicated in the aggregation process. Developmentally regulated lectins have been detected in embryonic chick pectoral muscle and differentiating L6 myoblasts in culture (4,5), in developing neonatal rat brain (6), and a β-D-galactoside-specific lectin has been purified from differentiating myoblasts (7). Lectins and complementary carbohydrates also may mediate bacterial cell adherence to host tissues. Adherence of Vibrio cholerae to intestinal brush border is inhibited by glycosides of

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L-fucose and D-mannose (8); D-mannose blocks Escherichia coli adherence to human oral mucosal cells (9), and the K88 adhesin of E. coli has specificity for tissue glycoproteins and glycolipids possessing heterosaccharide sequences terminating in β-D-galactosyl residues (10).

Pathogenicity of the cariogenic human oral bacterium Streptococcus mutans is dependent upon the adherence of bacterial cells to the tooth surface. The adherence of S. mutans cells to the host involves synthesis of dextrans from sucrose by exocellular bacterial dextransucrases (EC 2.4.1.5) and the concomitant binding of the bacterial cells to the dextrans, which tenaciously adhere to the tooth surface (11). The mechanism by which S. mutans cells bind dextran is not known, but several studies suggest the presence of a specific dextran-binding protein at the cell surface (12-15). We report here the purification of an extracellular dextran-binding protein, that is probably implicated in adherence of S. mutans to the host.

Materials and Methods

Streptococcus mutans 6715-49 (obtained from the Dental Research Unit, VA Hospital, Miami, Florida) was grown in glucose-supplemented cultures as previously described (16). A crude protein precipitate was prepared from cell-free 18 hr culture liquor by 50% saturation with $(NH_4)_2SO_4$ at 2°C, collected by centrifugation at 12,000 Xg, 2°C, for 20 min. and dissolved in 0.02% NaN_3.

A 500 ml aliquot of the crude protein preparation was applied to a 2.6 X 31 cm Sephadex G50 (Pharmacia Fine Chemicals, Piscataway, N.J) column equilibrated with 0.02% NaN_3. The charged column was washed overnight with 0.02% NaN_3. The column was then irrigated with 6M guanidine HCl and 2 ml fractions collected. Dextransucrase activity (Peak 1, Figure 1) was eluted before the guanidine HCl front and a second dextransucrase component was eluted with the guanidine HCl front. Fractions containing guanidine HCl (Figure 1, peak 2) were dialyzed against 0.02% NaN_3 and pooled.

An aliquot (5 ml) of peak 2 protein from the Sephadex G50 column was applied to a 2.6 X 86 cm column of Sepharose 4B (Pharmacia) and eluted with 0.02% NaN_3. Column effluent was monitored at 280 mμ and fractions of 2 ml were collected.

Dextransucrase activity was assayed in dialyzed column fractions by the procedure of McCabe and Smith (17). Contaminating traces of dextranase activity were detected in column fractions by incubating 100μl of each fraction with Sephadex G200 (1 ml settled volume) and 400μl 0.05M, pH 6.0 sodium phosphate buffer at 37°C for 1 hr. Supernatants were then assayed for solubilized carbohydrate by the method of Dubois, et al. (18). Protein in column fractions was assayed by the method of Lowry, et al. (19) following dialysis.

Reaction mixtures for assays of dextran binding by the purified dextran-binding protein (peak D, Figure 2) comprised 400μl purified binding protein (41μg protein); 100μl 0.5M, pH 6.0 sodium phosphate buffer or buffer containing clinical dextran (5 mg/ml); and 25μl [^{14}C]-dextran in 0.02% NaN_3 (137.5 μg,

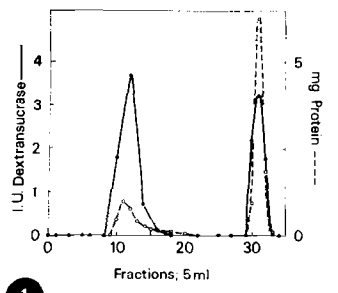
9727 CPM). In control reaction mixtures 400μl of 0.02% NaN_3 or solutions containing 48μg and 96μg of bovine serum albumin (BSA) were substituted for binding protein. Each reaction mixture was incubated for 30 min at 37°C and then filtered on a 25mm Millipore HAMK membrane filter (Millipore Corp., Bedford, Mass.). The membrane filter was washed with 10 ml of 0.08M, pH 6.0 sodium phosphate buffer, dried and the levels of [^{14}C]-dextran retained on the filters were determined by scintillation spectrometry. The [^{14}C]-dextran for this assay was synthesized with partially purified S. mutans 6715-49 dextransucrase (peak C, Figure 2) using clinical dextran (Nutritional Biochemicals, Cleveland, Ohio) as acceptor and [^{14}C]-([^{14}C]-U-glycosyl) sucrose (New England Nuclear, Boston, Mass.) as substrate. A reaction mixture (4 ml) comprising 12.7 mg dextran, 3.3 μCi [^{14}C]-sucrose, 6.7 mg sucrose (purified by passage through a dialysis membrane) and 0.012 International Units of dextransucrase and 125mM Tris acetate buffer, pH 6.0, was incubated at 37°C for 16 hrs. Reaction mixtures were heat-treated (30 min, 100°C) and centrifuged and [^{14}C]-dextran was precipitated from the supernatant solutions with 2 volumes of 95% ethanol, washed by thrice-repeated solubilization in water and precipitation with ethanol and finally dissolved in 1 ml 0.02% NaN_3.

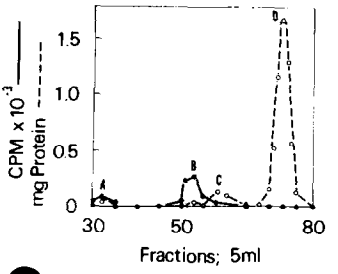
Polyacrylamide gel electrophoresis was by the method of Davis (20), omitting sample gels and stacking gels. Thin layer polyacrylamide gel electrofocussing was as described by Cowman (21) and SDS gel electrophoresis was by the method of Weber and Osborne (22). Proteins in gels were visualized by staining with Coomassie Brilliant Blue R250 (Bio Rad Laboratories, Rockville Centre, N.Y.); dextransucrase activity by incubation of gels in 1% sucrose or 1% sucrose plus 1.0 mg/ml clinical dextran in 0.5M Tris Acetate buffer, pH 6.0 (17).

Results and Discussion

The elution profiles for adsorbed protein and dextransucrase activity from the Sephadex G50 affinity chromatography column are shown in Figure 1. Unadsorbed material is not shown. Dextransucrase in Peak 1 was probably eluted from near the top of the charged column by the 6M guanidine HCl front and appeared in a protein fraction voided from the column (V_o after application of guanidine HCl). Peak 2, which coincided with emergence of the guanidine-HCl front, contained several major proteins, as revealed by electrophoresis (Figure 3). The predominant proteins in peak 2 formed a band containing dextransucrase activity and migrating 0.8 cm into the gel and a second band, devoid of dextransucrase activity, which migrated 3.2 cm into the gel (Figure 3). The use of dextran solutions instead of guanidine HCl also released a small portion of this latter protein from Sephadex G50 affinity columns (data not presented). Dextranase activity was detected in peak 1 but was absent from peak 2.

Gel filtration of peak 2 material on Sepharose 4B yielded 4 protein peaks (peaks A-D, Figure 2). Peaks A (V_o) and B contained dextransucrase activity,





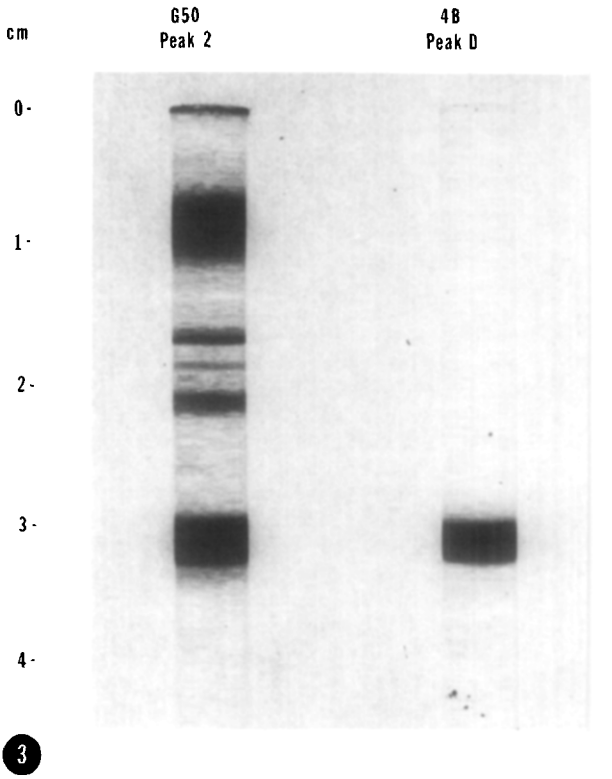


Figure 1. Affinity chromatography of a crude protein preparation (see Materials and Methods) on Sephadex G50. Material shown was retained during column washing and was subsequently eluted with 6M guanidine HCl.

Figure 2. Gel filtration of concentrated proteins from Sephadex G50 peak 2 (Figure 1, fractions 28-36) on Sepharose 4B eluted with 0.02% NaN_3.

Figure 3. Electrophoretograms of the proteins present in peak 2 eluted from the Sephadex G50 affinity chromatography column (Figure 1, fractions 23-36) and in peak D eluted from the Sepharose 4B gel filtration column (Figure 2, fractions 70-76). Staining by Coomassie brilliant blue R 250.

while peak C and peak D (V_t) were devoid of the enzyme activity. Protein of peak D formed a single zone upon polyacrylamide gel electrophoresis (Figure 3). Subsequent SDS gel electrophoresis and thin layer polyacrylamide gel electrofocussing of peak D material revealed a single protein zone, with an isoelectric point at pH 4.6. Dextran-binding protein (peak D) retained 4366 CPM of ^{14}C-dextran on the HAMK nitrocellulose membranes while controls retained from 177 CPM (NaN_3) to 276 CPM (96μg BSA) of ^{14}C-dextran. The presence of clinical

dextran in assay mixtures decreased ^{14}C-dextran retention by dextran-binding protein to 329 CPM.

The extracellular dextran-binding protein purified here has also been obtained in low yield from sonicates of washed S. mutans cells in which little or no cell rupture occurred, as indicated by the absence of intracellular markers in sonicate supernatants. It is likely, therefore, that the dextran-binding protein is a component of the bacterial cell surface, a possibility currently being explored. Data from numerous studies indicate the absolute requirement for bacterium-mediated dextran synthesis and the concomitant specific binding of dextran by S. mutans cells for establishment of this pathogen in the host (11). The purified dextran-binding protein of S. mutans provides a biochemical basis for specific cell adherence in this organism, and it represents the first lectin-like molecule purified from a bacterial pathogen, for which a role in host colonization and pathogenicity is indicated.

Acknowledgements

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References

1. Moscona, A.A. (1962) J. Cell. Comp. Physiol., 60, 65-80.
2. Simpson, D.L., Rosen, S.D. and Barondes, S.H. (1974) Biochem., 13, 3487-3493.
3. Simpson, D.L., Rosen, S.D. and Barondes, S.H. (1975) Biochim. Biophys. Acta, 412, 109-119.
4. Nowak, T.P., Haywood, P.L. and Barondes, S.H. (1976) Biochem. Biophys. Res. Comm., 68, 650-657.
5. Gartner, T.K. and Podleski, T.R. (1976) Biochem. Biophys. Res. Comm. 70, 1142-1149.
6. Simpson, D.L., Thorne, D.R. and Loe, H.H (1977) Nature, 266, 367-369.
7. Den, H. and Malinzak, D.A. (1976) Fed. Proc., 35, 1409 (Abstract 263).
8. Jones, G.W. and Freter, R. (1976) Infect. Immun., 14, 240-245.
9. Ofek, I., Mirelman, D. and Sharon, N. (1977) Nature, 265, 623-625.
10. Gibbons, R.A., Jones, G.W. and Sellwood, R. (1975) J. Gen. Microbiol., 86, 228-240.
11. Gibbons, R.J. and van Houte, J. (1973) J. Periodontol., 44, 347-360.
12. Spinell, D.M. and Gibbons, R.J. (1974) Infect. Immun., 10, 1448-1451.
13. Olson, G.A., Guggenheim, B. and Small, P.A. (1974) Infect. Immun., 9, 273-278.
14. Kelstrup, J. and Funder-Nielsen, T.D. (1974) J. Gen. Microbiol., 81, 485-489.

15. McCabe, M.M. and Smith, E.E. (1975) Infect. Immun., 12, 512-520.
16. McCabe, M.M. and Smith, E.E. (1973) Infect. Immun., 7, 829-838.
17. McCabe, M.M. and Smith, E.E. (1977) Infect. Immun., 16 (3), In Press.
18. Dubois, M., Gilles, K.A., Hamilton, J.K., Reber, P.A. and Smith, F. (1956) Anal. Chem., 28, 350-356.
19. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem., 193, 265-275.
20. Davis, B.J. (1974) Ann. N.Y. Acad. Sci., 12, 404-427.
21. Cowman, R.A., Fitzgerald, R.J. and Schaefer, S.J. (1976) Proceedings: "Microbial Aspects of Dental Caries", Eds, Stiles, Loesche, and O'Brien, Sp. Supp. Microbiology Abstracts, Vol II, 465-475.
22. Weber, K. and Osborn, M. (1969) J. Biol. Chem., 244, 4406-4412.