Vol. 149, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1987 Pages 86-92

THE SUGAR-SPECIFIC ADHESION/DEADHESION APPARATUS OF THE MARINE BACTERIUM VIBRIO FURNISSII IS A SENSORIUM THAT CONTINUOUSLY MONITORS NUTRIENT LEVELS IN THE ENVIRONMENT

Charles Yu, Andy M. Lee, and Saul Roseman

McCollum-Pratt Institute and Department of Biology,
Johns Hopkins University, Baltimore, Maryland 21218

Received October 7, 1987

SUMMARY: Our earlier studies on cell adhesion to immobilized carbohydrates are extended here to a marine bacterium, Vibrio furnissii. Apparently one lectin mediates the binding of these cells to glycosides of N-acetylglucosamine, mannose, and glucose covalently linked to Agarose beads. Kinetic studies show that protein synthesis is required for initiating and for **maintaining** adhesion to the glycosides. Furthermore, a pro$^-$ mutant binds to GlcNAc-beads at Pro concentrations insufficient to support cell growth. Expression of the functional lectin therefore predominates under conditions of limiting protein synthesis. Thus, cells adhere to the sugars in an environment compatible with protein synthesis, and deadhere when depleted of any required nutrient, presumably to migrate to a more favorable locale. The adhesion-deadhesion apparatus thereby permits constant monitoring of the surrounding environment, comprising a "nutrient sensorium".

The adhesion of bacteria to solid surfaces plays a key role in diverse pathological, ecological and biofouling phenomena and has been the subject of intense scrutiny for over two decades (1). Many bacterial genera utilize lectins to adhere to complex carbohydrates, such as those found on eukaryotic cell surfaces. Bacterial lectins are, however, specific for only a few sugars, most often for mannose or galactose, occasionally for sialic acid and L-fucose, and rarely for N-acetylglucosamine, N-acetylgalactosamine, or a glucan. Despite this extensive literature, there are few studies on bacterial adhesion to well defined immobilized carbohydrates (2-5) and there is apparently no information on the kinetics of bacterial cell adhesion to such sugars.

As shown below, a marine bacterium, Vibrio furnissii, expresses what appears to be a single lectin that binds to GlcNAc, Man, and

Abbreviations: 50% ASW, half concentrated artificial sea water supplemented with phosphate, ammonia, and buffer (composition given in Materials and Methods); AH, 6-aminohexanol.

0006-291X/87 $1.50

Glc. More importantly, continuous protein synthesis is required for the cells to **maintain** adhesion to the sugars. These results may have broad implications with respect to the physiological functions of bacterial lectin-sugar binding processes.

MATERIALS AND METHODS

Chitin oligosaccharides, glycosides, CCCP and monensin were obtained from the Sigma Chemical Co. (St. Louis, Mo.), D-[$U^{14}C$]-glucose from NEN (Boston, Mass.), L-[$U^{-14}C$]-amino acids from Amersham (Arlington Heights, Ill.), and [^{32}P]-Pi from ICN (Costa Mesa, CA). 6-Aminohexyl D-pyranosides were synthesized as reported (6), and were coupled to Affi-Gel 10 beads (Bio-Rad Co., Richmond, CA.) by the procedure described by the manufacturer; about 7 µmoles glycoside were covalently linked per g wet-packed beads.

V. furnissii 7225, kindly provided by P. Brayton and Dr. R. Colwell (Univ. of Maryland, College Park, Md.), was mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine (7,8) and 2 amino acid auxotrophs, a pro^- and a met^- mutant were isolated; they behaved similarly, and only results with V. furnissii 7225 and the pro-1 mutant (Strain CY21) are shown here. Cells were grown at pH 7.5, 25°C, with shaking, in "lactate-50% ASW" containing (g/l deionized water): D,L-lactate, 5; NaCl, 11.8; Na_2SO_4, 2.0; $NaHCO_3$, 0.1; KCl, 0.33; KBr, 0.048; H_3BO_3, 0.013; $MgCl_2,6H_2O$, 5.3; $SrCl_2,6H_2O$, 0.02; $CaCl_2,2H_2O$, 0.74; K_2HPO_4, 0.02; NH_4Cl, 1.0; 50 mM Hepes buffer. Growth was followed by measuring turbidity at 540 nm, viable cell counts, and by direct microscopic counting. Generation times in 50% ASW were: V. furnissii 7225, peptone-yeast extract, 1.0 h; lactate, 1.5 h; CY21, peptone-yeast extract, 1.0 h; CY21, lactate plus 200 µg Pro/ml, 1.5 h, plus 20 µg Pro/ml, 3.0 h. Cells were radiolabeled by growth in lactate-50% ASW containing 5 µCi ^{32}Pi, giving specific activities of about 0.02 cpm per cell. Only traces of ^{32}P were removed from the cells by repeated washing or after suspension in 50% ASW for 6 hr, at 25°C.

Adhesion Assay---Labeled, mid-log cells, 0.5-1 x 10^7 in 0.1 ml, were transferred from the cultures to 10 mg packed beads in 5 ml tubes, or the cells were first washed and resuspended in the desired medium. Centrifugation and washing did not affect cell-bead adhesion. After incubation at 22°C, 4.25 ml of 50% ASW were added to each tube, and the contents were gently poured on a 35 µm Nitex (Nylon) filter (Tetko Inc., Elmsford, N.Y.). The filters, containing the beads and labeled adherent cells, were washed twice, and counted. These values were corrected for cell-free controls (corresponding to 0.025 on the ordinates in the Figs.). In these experiments, the specific activities of the cells remained constant because ^{32}Pi was included in incubation mixtures containing Pi.

RESULTS

The model system employed here is a modification of that previously used to study the binding of tissue cultured cells, hepatocytes, and Dictyostelium discoideum, to immobilized, synthetic carbohydrates (9-13). 6-Aminohexyl-GlcNAc, -Man, -Glc, -Gal and the aglycon, 6-aminohexanol (AH), were covalently linked to activated Agarose beads. The beads were exposed to labeled V. furnissii cells, and the number of cells adhering to the beads determined.

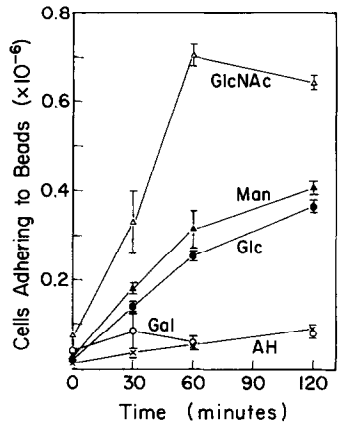

Fig. 1. **Adhesion of V. furnissii to glycoside-derivatized beads.** Adhesion was measured by the standard assay procedure in lactate-50% ASW (+^{32}Pi) growth medium. Each point is the average of duplicate incubations (vertical bars).

Control experiments showed that: (a) Identical results were obtained independent of the method used for labeling the cells (D-[U-^{14}C]-glucose, L-(U-^{14}C-amino acids, or [^{32}P]Pi). (b)Washed cells of the wild type strain maintained their adhesive properties when stored on ice for at least 2 h. (c) The number of sugar binding sites (on the beads) was not limiting, whereas the number of adherent cells was proportional to the quantity of cells employed. About 20% of the cells adhered to the beads under optimal conditions. (d) Thio- and O-glycoside-beads gave similar results.

Sugar Specificity---In lactate-50% ASW, V. furnissii cells bind most rapidly to GlcNAc, but also to Glc and Man (Fig. 1). However, about 20% of all cell preparations over an 18 month period showed equal affinities for the three sugars. The cells showed little to no adhesiveness to Gal-beads, even when grown on galactose as the sole carbon source.

A variety of sugars inhibited binding of the cells to the beads. In experiments with GlcNAc-beads, GlcNAc per se, and GlcNAc oligosaccharides and glycoside derivatives were the most potent inhibitors (>90% inhibition at 0.1 mM). At 10 mM concentrations, inhibition was also observed with Glc, 2-dGlc, methyl α-Glc, Man, cellobiose, sucrose, trehalose, L-fucose, arabinose, talose, idose, and gulose, but not with Gal and D-fucose.

To determine whether adhesion to the 3 sugars was mediated by one broadly specific or by as many as 3 mono-specific lectins, binding to GlcNAc-, Man- and Glc-beads was measured in the presence of 1 to 10^3 μM N,N',N'',N'''-tetraacetylchitotetraose. Essentially

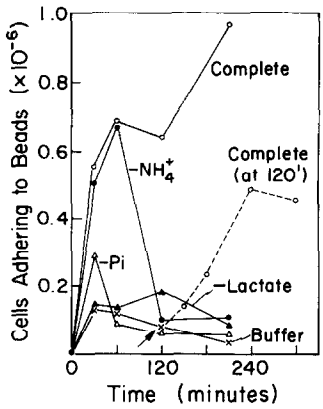

Fig. 2. **Requirements for adhesion of V. furnissii 7225 to GlcNAc-beads.** Cells were labeled, washed and stored in ice cold 50% ASW and assays conducted as described in "Methods". The symbols are as follows: ○ , complete medium (control); ● , NH_4Cl omitted; △ , Pi omitted; ▲ , lactate omitted; × , NH_4Cl, Pi, and lactate omitted. At the time shown by the arrow, NH_4Cl, Pi, and lactate were added (○--○).

the same results were obtained with the 3 sugar-beads, e.g., >90% inhibition at 0.1 mM tetraose. Furthermore, in each case, the tetraose was equally effective in preventing cell adhesion or in causing deadhesion of previously bound cells.

These results lead to the tentative conclusion that V. furnissii expresses a lectin which binds to GlcNAc, to Man, and to Glc, and which may well have even broader specificity.

The Requirement for Protein Synthesis---Wild type cells were grown in lactate-50% ASW, washed and stored **in the cold.** When the kinetics of adhesion to GlcNAc-beads were followed at 22°C, unexpected results were obtained (Fig. 2): Adhesion depended on the medium in which the cells and beads were suspended. In complete growth medium, the number of adherent cells increased with time. By sharp contrast, when ammonia or Pi were omitted, the cells adhered for a brief period, but promptly **deadhered.** While few cells adhered initially in lactate-free medium or in buffer alone, they rapidly adhered to the beads when supplemented with complete medium (120 min, Fig. 2).

Additional kinetic studies gave the following results: (a) Fixed cells (glutaraldehyde or formaldehyde) did not bind to the beads, and when formaldehyde was added after cell binding to GlcNAc-beads, the bacteria immediately deadhered. (b)Deadhesion did not result from splitting of the sugar ligand from the beads by glycosidases. Neither the addition of more beads nor the use

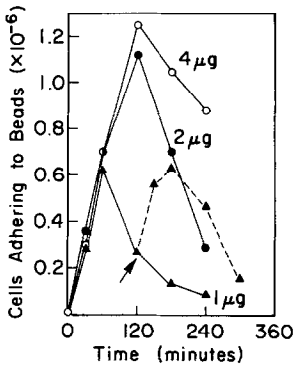

Fig. 3. **Proline is required for initiating and maintaining adhesion of CY21 (pro⁻) to GlcNAc-beads.** The cells were grown and labeled in lactate-50% ASW supplemented with Pro, harvested and washed in the cold with 50% ASW. Each adhesion assay mixture contained 0.5% lactate, 0.1% NH_4Cl, 0.002% K_2HPO_4 (5 μCi ^{32}Pi per ml) and the following quantities of L-Pro (μg/ml): ○ , 4.0; ● , 2.0; ▲ , 1.0. An additional 1.0 μg/ml of Pro was added at the arrow (▲--▲).

of thioglycosides, which are not cleaved by glycosidases, affected the results. (c)Metabolic poisons such as 1 mM cyanide (or 50 μM CCCP and monensin) inhibited adhesion. If the inhibitors were added following cell-bead adhesion (in complete medium), the cells rapidly deadhered. Protein synthesis inhibitors varied in their potency. (d) In otherwise complete medium, both the pro⁻ and met⁻ mutants required the respective amino acid for adhesion.

Fig. 3 shows some key results. When a limiting quantity of Pro was added (1-2 μg/ml), CY21 cells (pro⁻) adhered, and then deadhered. Deadhesion commenced when the Pro concentration fell below 0.7 μg/ml (as determined with labeled Pro). When 1 μg Pro/ml was added following deadhesion (120 min), the cells once again bound to the GlcNAc-beads.

DISCUSSION

V. furnissii apparently expresses an unusual lectin, with broader sugar-specificity than heretofore reported for bacterial lectins. However, the kinetics reported here are of even greater interest since they suggest how the lectin may function.

All of our data support the conclusion that protein synthesis is required not only to initiate cell adhesion to sugars, but to **maintain** this bond. Several recent reports suggest that protein synthesis is required for lectin-mediated adhesion of bacterial cells. This conclusion was usually derived by using sub-lethal concentrations of antibiotics during growth of the organism (14-

20). In a few of these experiments, inhibitors were tested directly during the adhesion assay. There are apparently no studies showing that continuous protein synthesis is necessary for the bacteria to maintain cell-sugar binding. This requirement by V. furnissii is independent of cell division, since: (a) Adhesion of strain CY21 to GlcNAc-beads proceeds without a lag at 1 µg Pro/ml, a concentration far below that required for cell division (20 µg/ml). (b)The generation time in 20 µg Pro/ml is 3 h, whereas adhesion/deadhesion occurs in a much shorter time span.

Our results imply that the functional (cell surface) lectin turns over more rapidly than whole cell protein, and that the lectin is preferentially expressed since adhesion occurs at Pro levels insufficient to support growth. These speculations make teleological sense. That is, V. furnissii attaches to the sugar substratum when conditions are favorable for at least minimal protein synthesis (expression of functional lectin), and remains attached as long as the environment contains nutrients required for protein synthesis. When the medium is depleted of even one nutrient (Pi, NH_4^+, lactate or Pro in the case of CY21), the cells deadhere, and presumably migrate toward a favorable environment.

We suggest that lectin synthesis, expression, and turnover, associated with cell adhesion/deadhesion to immobilized sugars by V. furnissii, is a complex phenomenon comprising a **nutrient sensorium**, which constantly monitors the surrounding environment.

ACKNOWLEDGEMENTS

*This work was supported by Contract No. N0001485-K-0072 from the Office of Naval Research. This is contribution #1379 from the McCollum-Pratt Institute. We are especially grateful to Drs. Philip Hartman and Jay Grimes for their many valuable suggestions.

REFERENCES

1. Mirelman, D. (1986) Microbial Lectins and Agglutinins: Properties and Biological Activity, Wiley Series in Ecological and Applied Microbiology (Series Ed., Mitchell, R.), Wiley Interscience, New York.
2. Jones, G.W., and Freter, R. (1976) Infect. Immun. 14, 240-245
3. Svenson, S.B., Hultberg, H., Källenius, G., Korhonen, T.K., Möllby, R. and Winberg, J. (1983) Infect. 11, 73-78
4. Hansson, G.C., Karlsson, K.A., Larson, G., Lindberg, A., Strömberg, N., and Thurin, J., in Proc. 7th Int. Symp. Glycoconjugates, Lund (1983) (Chester, M.A., Heinegard, D., Lundblad, A., and Svensson, S., eds) p. 631 Rahms, Lund, Sweden
5. Svenson, S.B., Källenius, G., Möllby, R., Hultberg, H., and Winberg, J. (1982) Infect. 10, 209-214

6. Chipowsky, S., and Lee, Y.C. (1973) Carbohydr. Res. 31, 339-346
7. Adelberg, E.A., Mandel, M., Chen, G.C.C. (1965) Biochem. Biophys. Res. Commun. 18, 788-795
8. Carlton, B.C., and Brown, B.J. in (1981) Manual of Methods for General Bacteriology (Gerhardt, P., and Nester, E.W., eds) Pp. 222-242 Americal Soc. for Microbiol., Washington, D.C.
9. Chipowsky, S., Lee, Y. C., and Roseman, S. (1973) Proc. Nat. Acad. Sci. (USA)., 70, 2309-2312
10. Schnaar, R. L., Weigel, P. H., Kuhlenschmidt, M. S., Lee, Y. C. and Roseman, S. (1978) J. Biol. Chem. 253, 7940-7951
11. Weigel, P. H., Schnaar, R. L., Kuhlenschmidt, M. S., Schmell, E., Lee, R. T., Lee, Y. C., and Roseman, S. (1979) J. Biol. Chem. 254, 10830-10838
12. Pless, D. D., Lee, Y. C., Roseman, S. and Schnaar,R. L. (1983) J. Biol. Chem. 258, 2340-2349
13. Bozzaro, S. and Roseman, S. (1983) J. Biol. Chem. 258, 13882-13889, 13890-13897
14. Ofek, I., Beachey, E.H., Eisenstein, B.I., Alkan, M.L., and Sharon, N. (1979) Rev. Infect. Dis. 1, 832-837
15. Beachey, E.H., Eisenstein, B.I., and Ofek, I. (1982) in The Influence of Antibiotics on the Host-Parasite Relationship (Eickenberg, H.-V., Hahn, H., and Opferkuch, W., eds.) p. 171 Springer-Verlag, Germany
16. Vosbeck, K., Mett, H., Huber, U., Bohn, J., and Petignat, M. (1982) Antimicrobial Agents and Chemotherapy 21, 864-869
17. Sandberg, T., Stenqvist, K., and Svanborg-Edèn (1979) Rev. Infect. Dis. 1, 838-844
18. Chopra, I., and Hacker, K. (1986) J. Antimicrobial Chemotherapy 18, 441-451
19. Paul, J.H. (1984) Appl. Envir. Microbiol. 48, 924-929
20. Shibl, A.M. (1985) Rev. Infectious Dis. 7, 51-65