SHORT COMMUNICATION

Interactions of *Pseudomonas aeruginosa* Lectins with *Escherichia coli* Strains Bearing Blood Group Determinants

By N. GARBER,* J. GLICK, N. GILBOA-GARBER AND A. HELLER

Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

(Received 11 September 1980; revised 17 November 1980)

Pseudomonas aeruginosa lectins interact with *Escherichia coli* strains O₈₆B₇ and O₁₂₈B₁₂, which possess B and H (O) blood group determinants, respectively. The interaction could be demonstrated by specific agglutination of the bacteria, by haemagglutination inhibition tests and by lectin-mediated peroxidase binding to the bacteria. The agglutination of *E. coli* O₈₆B₇ by the *Pseudomonas* galactose-binding lectin was inhibited by D-galactose and by the lipopolysaccharide extracted from *E. coli* O₈₆B₇. Similarly, the specific agglutination of *E. coli* O₁₂₈B₁₂ by the *Pseudomonas* mannose-binding lectin (which also binds L-fucose, L-galactose and D-fructose) was inhibited by D-mannose, L-fucose, L-galactose and D-fructose, as well as by the lipopolysaccharide extracted from *E. coli* O₁₂₈B₁₂. The interaction between *E. coli* O₁₂₈B₁₂ and the *Pseudomonas* mannose-binding lectin was also demonstrated by lectin-mediated peroxidase binding to the bacterial surface. Peroxidase binding was also inhibited by the above-mentioned sugars and *E. coli* O₁₂₈B₁₂ lipopolysaccharide.

Treatment of cells of the two *E. coli* strains with protein-denaturing agents did not reduce their agglutination by the *Pseudomonas* lectins. On the other hand, oxidation of the cell surface sugars by sodium metaperiodate or boiling the cells in the presence of 1% acetic acid for 1 h abolished their agglutination by the two lectins. It is, therefore, suggested that the *Pseudomonas* lectins interact with the B and H (O) blood group determinant sugars (D-galactose in *E. coli* O₈₆B₇ and L-fucose in *E. coli* O₁₂₈B₁₂) residing in the lipopolysaccharides of these *E. coli* strains.

INTRODUCTION

Lectins, due to their sugar-binding specificity, are important tools for investigating cell surface receptors in higher organisms (Nicolson, 1974; Lis & Sharon, 1977; Goldstein & Hayes, 1978), protozoa (Martinez-Palomo *et al.*, 1973) and bacteria (Gilboa-Garber *et al.*, 1977*b*; Le Minor *et al.*, 1973; Lotan *et al.*, 1975). The interaction of plant lectins with certain micro-organisms made them useful for bacterial typing (Le Minor *et al.*, 1973), for characterizing bacterial cell components (Archibald & Coapes, 1971; Goldstein & Misaki, 1970; Lotan *et al.*, 1975) and for determining bacteriophage receptors (Archibald & Coapes, 1972). Bacterial lectins, which resemble the plant lectins in sugar specificity, relative thermostability, divalent cation requirement and some other properties (Gilboa-Garber *et al.*, 1977*a*), may be employed for the same purposes (Gilboa-Garber *et al.*, 1977*b*). In the present study two *Pseudomonas* lectins derived from bacterial cell extracts (not from fimbriae) were used. One lectin binds D-galactose and its derivatives (Ps-GAL) (Gilboa-Garber *et al.*, 1972) and the other binds D-mannose, L-fucose, L-galactose and D-fructose (Ps-MAN) (Gilboa-Garber *et al.*, 1977*a*). The interaction of these two lectins of *Pseudomonas aeruginosa* with *Escherichia coli* strains O₈₆B₇ and O₁₂₈B₁₂ was analysed. The O antigen of *E. coli* O₈₆B₇ is

known to react with anti-B serum and that of *E. coli* O₁₂₈B₁₂ exhibits an H (O) blood group specificity (Springer, 1971). Since the determinants of the O antigens of the two bacteria contain D-galactose and L-fucose, respectively, the possibility that a specific interaction of the lectins with these bacteria may be related to their O antigen specificity was considered. Interaction of plant lectins with bacterial O-antigen-containing LPS has been described (Wolpert & Albersheim, 1976).

METHODS

Bacterial strains. *Escherichia coli* O₈₆B₇ and *E. coli* O₁₂₈B₁₂ were isolated during a survey of strains of enteropathogenic *E. coli* in Israel (Garber & Frimerman, 1973).

***Pseudomonas* lectins.** The two *Pseudomonas aeruginosa* lectins were prepared by heating cell extracts to 70 °C for 15 min, followed by ammonium sulphate precipitation (60% saturation) and affinity chromatography using Sepharose 4B, as previously described (Gilboa-Garber *et al.*, 1972, 1977a).

Bacterial suspensions. Bacteria were grown at 37 °C with shaking in nutrient broth (Difco) for 6 h (exponential phase). They were then harvested, washed three times and resuspended (5% wet w/v) in 0.9% NaCl (saline).

Lipopolysaccharide preparations. Lipopolysaccharide (LPS) preparations of *E. coli* O₁₂₈B₁₂ and O₈₆B₇ were prepared according to Westphal & Jann (1965). Hydrolysed LPS was prepared by treatment with 1 M-HCl at 105 °C for 4 h in sealed ampoules (Wiseman & Caird, 1977) followed by neutralization with solid BaCO₃ and centrifugation at 27000 g for 30 min; the clear solution was lyophilized and kept at -20 °C.

Agglutination tests. Portions (0.05 ml) of the 5% bacterial suspensions or of a 5% suspension of papain-treated human erythrocytes (Gilboa-Garber, 1972) were added to 0.2 ml portions of the *Pseudomonas* lectin preparations (initial concentration 0.5 mg protein ml⁻¹) diluted in a series of two-fold dilutions in saline. After incubation at room temperature for 30 min (for haemagglutination) or 2 h (for bacterial agglutination), the agglutination titre was determined visually (Gilboa-Garber *et al.*, 1977b); titres are expressed as the reciprocal of the highest dilution that showed agglutination.

Inhibition of bacterial agglutination and haemagglutination by sugars and LPS from *E. coli* O₁₂₈B₁₂ and O₈₆B₇. The Ps-MAN or Ps-GAL preparations (0.2 ml) were mixed with 0.2 ml of sugar solution, or LPS from the *E. coli* strains or HCl-hydrolysed LPS from the same strains, each at the concentration specified in Table 1. After 1 h incubation at room temperature, a series of two-fold dilutions was made in 0.2 ml saline (final volume); bacteria or papain-treated erythrocytes were then added as described in the agglutination tests. The sugars used were D-mannose, D-galactose, L-galactose and D-fructose (Sigma) and L-fucose (Fluka AG).

Adsorption test. To test for adsorption of the *Pseudomonas* lectins on to the bacterial cells, 0.5 g of thrice-washed exponential phase packed bacterial cells was incubated with 1 ml of the *Pseudomonas* lectin (0.5 mg protein ml⁻¹) at room temperature for 1 h. The supernatant obtained after removal of the bacteria was examined for residual haemagglutinating activity, as described in the agglutination tests.

Peroxidase binding. Peroxidase binding to *E. coli* O₁₂₈B₁₂ mediated by *Pseudomonas* lectins was assessed as previously described (Gilboa-Garber *et al.*, 1977a). Bacteria were incubated for 15 min with Ps-MAN and horseradish peroxidase. After thorough washing of the cells the bound peroxidase was eluted with 0.1 M-D-mannose solution and determined by *o*-dianisidine and H₂O₂.

Various treatments of the intact *E. coli* cells. Cells of *E. coli* O₈₆B₇ and O₁₂₈B₁₂ were subjected to the following treatments: ethanol (25%, v/v) and formaldehyde (0.5%, v/v) each for 18 h at room temperature; storage for 1 month at 4 °C; osmotic shock according to Neu & Heppel (1965); heating at 100 °C for 1 h in the presence or absence of 1% (v/v) acetic acid in saline; oxidation by sodium metaperiodate. Oxidation by sodium metaperiodate was performed according to Sabet & Schnaitman (1971) with the following modifications: 0.35 ml of the bacterial suspension (5% wet w/v) in saline was incubated with 0.15 ml containing 10 µmol Tris/HCl buffer pH 7 and 20 µmol sodium metaperiodate (Merck) in the dark at room temperature for 1 h; the treatment was stopped by the addition of D-glucose solution at a final concentration of 5% (w/v). After each treatment the cells were washed three times with saline and resuspended to a concentration of 5%.

Papain or pronase (Sigma, 0.15% w/v) were used for proteolytic digestions. Papain treatment was performed in 0.01 M-Tris/HCl buffer pH 7 containing 1.5 mg L-cysteine ml⁻¹ (Sigma) at 37 °C for 30 min. Pronase digestion was done in 0.01 M-Tris/HCl buffer pH 8 containing 0.01 M-CaCl₂ at 37 °C for 30 min.

Analytical procedures for LPS. Total carbohydrates were determined by the method of Dubois *et al.* (1956). 2-Keto-3-deoxyoctonic acid (KDO) was determined by the method of Karkhanis *et al.* (1978). Total phosphate was determined by the method of Ames (1966) and total protein by the Lowry method.

RESULTS

Ps-GAL agglutinated *E. coli* O₈₆B₇, but not *E. coli* O₁₂₈B₁₂. The agglutination was partially inhibited by *E. coli* O₈₆B₇ LPS and completely inhibited by HCl-hydrolysed LPS of this

Table 1. Agglutination of *E. coli* strains and papain-treated human red blood cells by the *Pseudomonas* lectins before and after their exposure to simple sugars, to *E. coli* LPS and intact cells

Addition*	Bacterial agglutination titre		Haemagglutination titre	
	Ps-GAL/ <i>O</i> ₈₆ B ₇	Ps-MAN/ <i>O</i> ₁₂₈ B ₁₂	Ps-GAL	Ps-MAN
None	32	32	512	128
D-Mannose	32	0	512	0
D-Galactose	0	32	0	128
L-Fucose	32	0	512	0
D-Fructose	32	0	512	0
L-Galactose	32	0	512	0
LPS <i>O</i> ₈₆ B ₇ †	8	32	—	—
LPS <i>O</i> ₈₆ B ₇ (HCl-hydrolysed)	0	—	—	—
LPS <i>O</i> ₁₂₈ B ₁₂ †	32	0	—	—
LPS <i>O</i> ₁₂₈ B ₁₂ (HCl-hydrolysed)	—	0	—	—
<i>E. coli</i> <i>O</i> ₁₂₈ B ₁₂	—	—	512	0
<i>E. coli</i> <i>O</i> ₈₆ B ₇	—	—	32	64

—, Not tested.

* Potential inhibitors were added at the following final concentrations. (a) Ps-GAL/*O*₈₆B₇ agglutination: simple sugars, 150 mM, except L-galactose, 30 mM; LPS, 12.5 mg ml⁻¹; HCl-hydrolysed LPS, 1.25 mg ml⁻¹. (b) Ps-MAN/*O*₁₂₈B₁₂ agglutination: simple sugars, 150 mM, except L-galactose, 30 mM; LPS, 0.5 mg ml⁻¹; HCl-hydrolysed LPS, 5 mg ml⁻¹. (c) Ps-GAL haemagglutination: simple sugars, 300 mM, except D-galactose, 10 mM. (d) Ps-MAN haemagglutination: D-mannose, 3 mM; D-galactose, 300 mM; L-fucose, 0.5 mM; D-fructose and L-galactose, 30 mM.

† Composition analysis of the LPS obtained from *E. coli* *O*₈₆B₇ and *O*₁₂₈B₁₂, respectively (% w/w): total carbohydrates, 36.3 and 16.3; KDO, 4.4 and 3.5; total phosphate, 2.7 and 0.7; protein, 0.5 and 2.4.

bacterium. The LPS of *E. coli* *O*₁₂₈B₁₂ did not inhibit *E. coli* *O*₈₆B₇ agglutination (Table 1). Ps-MAN (which binds D-mannose, L-fucose, L-galactose and D-fructose) agglutinated *E. coli* *O*₁₂₈B₁₂, but not *E. coli* *O*₈₆B₇. Addition of D-mannose, L-fucose, L-galactose and D-fructose, but not D-galactose, inhibited *E. coli* *O*₁₂₈B₁₂ agglutination. Both HCl-hydrolysed and untreated LPS of *E. coli* *O*₁₂₈B₁₂ inhibited *E. coli* *O*₁₂₈B₁₂ agglutination, whereas the LPS of *E. coli* *O*₈₆B₇ did not (Table 1).

Washed cells of *E. coli* *O*₈₆B₇ and *O*₁₂₈B₁₂ adsorbed Ps-GAL and Ps-MAN, respectively, reducing the haemagglutination titre of the lectin preparations towards papain-treated human erythrocytes (Table 1). The reduction of the haemagglutinating activity was comparable with that observed with the respective sugars (Table 1).

Ps-MAN-mediated binding of horseradish peroxidase to *E. coli* *O*₁₂₈B₁₂ was observed. D-Mannose, D-fructose, L-galactose, L-fucose (each at 100 mM) and *E. coli* *O*₁₂₈B₁₂ LPS (800 µg ml⁻¹) inhibited this binding by 84 to 92%, whereas D-galactose (100 mM) and *E. coli* *O*₈₆B₇ LPS (800 µg ml⁻¹) were not inhibitory.

Treatment of *E. coli* *O*₈₆B₇ and *O*₁₂₈B₁₂ cells with formaldehyde, ethanol, papain or pronase, storage for 1 month at 4 °C or osmotic shock did not affect their agglutinability by Ps-GAL and Ps-MAN. Boiling in saline for 10 or 60 min even increased the agglutination of these bacteria by the two *Pseudomonas* lectins. Abolition of the cell agglutinability was obtained only by boiling the bacteria in 1% acetic acid for 1 h or by metaperiodate oxidation.

DISCUSSION

The agglutination of *E. coli* *O*₈₆B₇ cells by Ps-GAL and its inhibition by D-galactose and by the LPS of this strain (Table 1) indicate that Ps-GAL interacts with the LPS of this bacterium which bears D-galactose (Orskov *et al.*, 1967) in an O-α-D-Gal(1-3)-β-D-Gal terminal residue. This residue is known to possess B blood group specificity (Springer, 1971). Since Ps-GAL is not strictly specific to α-derivatives of terminal D-galactose, but reacts also with β-D-galactosides and other D-galactose derivatives present on red blood cells, it is not blood

group specific in haemagglutination. The agglutination of *E. coli* O₁₂₈B₁₂ by Ps-MAN and its inhibition by D-mannose, L-fucose, D-fructose, L-galactose and by the LPS of this strain (Table 1) may indicate that Ps-MAN interacts with the L-fucose which resides in the LPS of this bacterium (Orskov *et al.*, 1967) and contributes to its H (O) blood group specificity (Springer, 1971). This LPS also contains D-galactose in its structure (Orskov *et al.*, 1967) but there was no interaction of this sugar with Ps-GAL. The results of the inhibition of haemagglutination by sugars and of Ps-MAN-mediated binding of peroxidase to *E. coli* O₁₂₈B₁₂ were similar to those of bacterial agglutination. The resistance of the lectin receptors in the *E. coli* strains to protein-denaturing agents indicates that the bacterial receptors involved in the interaction are not protein in nature. On the other hand, the sensitivity of the agglutination to oxidation by metaperiodate (West *et al.*, 1967) and to hydrolysis by acetic acid (Hammarstrom *et al.*, 1972) is compatible with the interpretation that the receptors of the *E. coli* strains for the *Pseudomonas* lectins are part of their LPS.

The authors wish to thank Mrs Aviva Belz and Miss Lea Mizrahi for skilful technical assistance, Miss Esther Guggenheimer for the revision, and Mrs Bluma Lederhendler for typing the manuscript. This study is part of the Ph.D. thesis of J. Glick, to be submitted to Bar-Ilan University, Ramat-Gan, Israel.

REFERENCES

- AMES, B. N. (1966). Assay of inorganic phosphate, total phosphate and phosphatases. *Methods in Enzymology* **8**, 115–118.
- ARCHIBALD, A. R. & COAPES, H. E. (1971). The interaction of Concanavalin A with teichoic acids and bacterial walls. *Biochemical Journal* **123**, 665–667.
- ARCHIBALD, A. R. & COAPES, H. E. (1972). Blocking of bacteriophage receptor sites by Concanavalin A. *Journal of General Microbiology* **73**, 581–585.
- DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350–356.
- GARBER, N. & FRIMERMAN, H. (1973). Resistance of enteropathogenic strains of *Escherichia coli* to penicillins and cephalosporins. *Israel Journal of Medical Sciences* **9**, 888–894.
- GILBOA-GARBER, N. (1972). Inhibition of broad spectrum haemagglutinin from *Pseudomonas aeruginosa* by D-galactose and its derivatives. *FEBS Letters* **20**, 242–244.
- GILBOA-GARBER, N., MIZRAHI, L. & GARBER, N. (1972). Purification of the galactose-binding haemagglutinin of *Pseudomonas aeruginosa* by affinity chromatography using Sepharose. *FEBS Letters* **28**, 93–95.
- GILBOA-GARBER, N., MIZRAHI, L. & GARBER, N. (1977a). Mannose-binding haemagglutinins in extracts of *Pseudomonas aeruginosa*. *Canadian Journal of Biochemistry* **55**, 975–981.
- GILBOA-GARBER, N., NIR-MIZRAHI, I. & MIZRAHI, L. (1977b). Specific agglutination of *Escherichia coli* O₁₂₈B₁₂ by the mannose-binding proteins of *Pseudomonas aeruginosa*. *Microbios* **18**, 99–109.
- GOLDSTEIN, I. J. & HAYES, C. E. (1978). The lectins: carbohydrate-binding proteins of plants and animals. *Advances in Carbohydrate Chemistry and Biochemistry* **35**, 127–340.
- GOLDSTEIN, I. J. & MISAKI, A. (1970). Interaction of Concanavalin A with arabinogalactan from the cell wall of *Mycobacterium bovis*. *Journal of Bacteriology* **103**, 422–425.
- HAMMARSTROM, S., LINDBERG, A. A. & ROBERTSSON, E. S. (1972). Precipitation of lipopolysaccharides from rough mutants of *Salmonella typhimurium* by an A-hemagglutinin from *Helix pomatia*. *European Journal of Biochemistry* **25**, 274–282.
- KARKHANIS, Y. D., ZELTNER, J. V., JACKSON, J. J. & CARLO, D. J. (1978). A new and improved micro-assay to determine 2-keto-3-deoxyoctonate in lipopolysaccharide of Gram-negative bacteria. *Analytical Biochemistry* **85**, 595–601.
- LE MINOR, L., TOURNIER, P. & CHALON, A. M. (1973). Agglutinabilité par la Concanavaline A de diverse bacilles à Gram-négatif. Etude chez les *Salmonella* de la corrélation avec la structure antigénique O. *Annales de Microbiologie* **124A**, 467–476.
- LIS, H. & SHARON, N. (1977). Lectins: their chemistry and application to immunology. In *The Antigens*, vol. 4, pp. 429–529. Edited by M. Sela. New York: Academic Press.
- LOTAN, R., SHARON, N. & MIRELMAN, D. (1975). Interaction of wheat-germ agglutinin with bacterial cells and cell-wall polymers. *European Journal of Biochemistry* **55**, 257–262.
- MARTINEZ-PALOMO, A., GONZALEZ-ROBLES, A. & DE LA TORRE, M. (1973). Selective agglutination of pathogenic strains of *Entamoeba histolytica* induced by Con A. *Nature New Biology* **245**, 186–187.
- NEU, H. C. & HEPPEL, L. A. (1965). The release of enzymes from *Escherichia coli* by osmotic shock and during the formation of spheroplasts. *Journal of Biological Chemistry* **240**, 3685–3692.
- NICOLSON, G. L. (1974). The interactions of lectins with animal cell surfaces. *International Review of Cytology* **39**, 89–190.
- ORSKOV, F., ORSKOV, I., JANN, B., JANN, K., MULLER-SEITZ, E. & WESTPHAL, O. (1967). Immunochemistry of *Escherichia coli* O antigens. *Acta pathologica et microbiologica scandinavica* **71**, 339–358.

- SABET, S. F. & SCHNAITMAN, C. A. (1971). Localization and solubilization of colicin receptors. *Journal of Bacteriology* **108**, 422–430.
- SPRINGER, G. F. (1971). Blood-group and Forssman antigenic determinants shared between microbes and mammalian cells. *Progress in Allergy* **15**, 9–77.
- WEST, E. S., TODD, W. R., MASON, H. S. & VAN BRUGGEN, J. T. (1967). *Textbook of Biochemistry*, 4th edn, p. 215. London: Collier-Macmillan.
- WESTPHAL, O. & JANN, K. (1965). Bacterial lipopolysaccharides. *Methods in Carbohydrate Chemistry* **5**, 83–91.
- WISEMAN, G. M. & CAIRD, J. D. (1977). Composition of the lipopolysaccharide of *Neisseria gonorrhoeae*. *Infection and Immunity* **16**, 550–556.
- WOLPERT, J. S. & ALBERSHEIM, P. (1976). Host-symbiont interactions. I. The lectins of legumes interact with the O-antigen-containing lipopolysaccharides of their symbiont rhizobia. *Biochemical and Biophysical Research Communications* **70**, 729–737.