SHORT COMMUNICATION

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

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(Received 11 September 1980; revised 17 November 1980)

Pseudomonas aeruginosa lectins interact with Escherichia coli strains O_{86},B_{7} and O_{128},B_{12}, 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 O_{86},B_{7} by the Pseudomonas galactose-binding lectin was inhibited by D-galactose and by the lipopolysaccharide extracted from E. coli O_{86},B_{7}. Similarly, the specific agglutination of E. coli O_{128},B_{12} 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 O_{128},B_{12}. The interaction between E. coli O_{128},B_{12} 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 O_{128},B_{12} 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 O_{86},B_{7} and L-fucose in E. coli O_{128},B_{12}) 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., 1977b; 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., 1977a), may be employed for the same purposes (Gilboa-Garber et al., 1977b). 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., 1977a). The interaction of these two lectins of Pseudomonas aeruginosa with Escherichia coli strains O_{86},B_{7} and O_{128},B_{12} was analysed. The O antigen of E. coli O_{86},B_{7} is
known to react with anti-B serum and that of E. coli O_{128}B_{12} 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 O_{66}B_{7} and E. coli O_{128}B_{12} 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 in 0.9% NaCl (saline).

Lipopolysaccharide preparations. Lipopolysaccharide (LPS) preparations of E. coli O_{128}B_{12} and O_{66}B_{7} 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 BaCO_{3} and centrifugation at 27 000 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 O_{128}B_{12} and O_{66}B_{7}. 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 O_{66}B_{12} 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-M-CaCl_{2} at 37 °C for 30 min. The eluate was examined for residual peroxidase activity, as described in the agglutination tests.

Various treatments of the intact E. coli cells. Cells of E. coli O_{66}B_{7} and O_{128}B_{12} 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-CaCl_{2} 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 O_{66}B_{7}, but not E. coli O_{128}B_{12}. The agglutination was partially inhibited by E. coli O_{66}B_{7} 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

<table>
<thead>
<tr>
<th>Addition*</th>
<th>Bacterial agglutination titre</th>
<th>Haemagglutination titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ps-GAL/O\textsubscript{66}B\textsubscript{7}</td>
<td>Ps-MAN/O\textsubscript{128}B\textsubscript{12}</td>
</tr>
<tr>
<td>None</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>L-Fucose</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>L-Galactose</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>LPS O\textsubscript{66}B\textsubscript{7} (HCl-hydrolysed)</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>LPS O\textsubscript{128}B\textsubscript{12}†</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>LPS O\textsubscript{128}B\textsubscript{12} (HCl-hydrolysed)</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>E. coli O\textsubscript{128}B\textsubscript{12}</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>E. coli O\textsubscript{66}B\textsubscript{7}</td>
<td>—</td>
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</tr>
</tbody>
</table>

* Potential inhibitors were added at the following final concentrations. (a) Ps-GAL/O\textsubscript{66}B\textsubscript{7} agglutination: simple sugars, 150 mM, except L-galactose, 30 mM; LPS, 12.5 mg ml\textsuperscript{-1}; HCl-hydrolysed LPS, 1.25 mg ml\textsuperscript{-1}. (b) Ps-MAN/O\textsubscript{128}B\textsubscript{12} agglutination: simple sugars, 150 mM, except L-galactose, 30 mM; LPS, 0.5 mg ml\textsuperscript{-1}; HCl-hydrolysed LPS, 5 mg ml\textsuperscript{-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\textsubscript{66}B\textsubscript{7} and O\textsubscript{128}B\textsubscript{12}, 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.

Washed cells of E. coli O\textsubscript{66}B\textsubscript{7} and O\textsubscript{128}B\textsubscript{12} 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\textsubscript{128}B\textsubscript{12} was observed. D-Mannose, D-fructose, L-galactose, L-fucose (each at 100 mM) and E. coli O\textsubscript{128}B\textsubscript{12} LPS (800 µg ml\textsuperscript{-1}) inhibited this binding by 84 to 92%, whereas D-galactose (100 mM) and E. coli O\textsubscript{66}B\textsubscript{7} LPS (800 µg ml\textsuperscript{-1}) were not inhibitory.

Treatment of E. coli O\textsubscript{66}B\textsubscript{7} and O\textsubscript{128}B\textsubscript{12} 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\textsubscript{66}B\textsubscript{7} 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 bacterium. The LPS of E. coli O\textsubscript{128}B\textsubscript{12} did not inhibit E. coli O\textsubscript{66}B\textsubscript{7} agglutination (Table 1). Ps-MAN (which binds D-mannose, L-fucose, L-galactose and D-fructose) agglutinated E. coli O\textsubscript{128}B\textsubscript{12}, but not E. coli O\textsubscript{66}B\textsubscript{7}. Addition of D-mannose, L-fucose, L-galactose and D-fructose, but not D-galactose, inhibited E. coli O\textsubscript{66}B\textsubscript{7} agglutination. Both HCl-hydrolysed and untreated LPS of E. coli O\textsubscript{128}B\textsubscript{12} inhibited E. coli O\textsubscript{128}B\textsubscript{12} agglutination, whereas the LPS of E. coli O\textsubscript{66}B\textsubscript{7} did not (Table 1).

Washed cells of E. coli O\textsubscript{66}B\textsubscript{7} and O\textsubscript{128}B\textsubscript{12} 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).
group specific in haemagglutination. The agglutination of E. coli O12B12 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 O12B12 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


