Inhibition of the Interaction Between Fimbrial Haemagglutinins and Erythrocytes by D-Mannose and Other Carbohydrates

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SUMMARY

Many monosaccharides, oligosaccharides and their derivatives were tested as inhibitors of agglutination of guinea-pig or horse erythrocytes by *Salmonella typhimurium* or *Shigella flexneri* bacteria bearing type-I fimbriae. D-Mannose and certain derivatives of mannose were strongly inhibitory, and D-fructose moderately inhibitory. Modification of any of the hydroxyl groups at the C-2, C-3, C-4 or C-6 positions in the D-mannopyranosyl molecule caused failure to inhibit, showing that these groups were necessary for binding to the active sites of the fimbrial haemagglutinin. The α-configuration at the C-1 position in D-mannose was important and carbohydrates containing α-linked D-mannose were much more active inhibitors than those containing β-linked D-mannose, which were poor inhibitors or non-inhibitors. Yeast mannan also inhibited the fimbrial reaction. Attention is drawn to the remarkable degree of similarity of the inhibition pattern of the type-I fimbrial haemagglutinin of bacteria with that of concanavalin-A, the jack bean haemagglutinin.

INTRODUCTION

The bacterial appendages called fimbriae by Duguid, Smith, Dempster & Edmunds (1955) have been classified as sex and common fimbriae (Duguid, Anderson & Campbell, 1966). The presence of common fimbriae on a bacterium is associated with adhesive properties in five of the six types recognized (Duguid et al. 1966). Only type-2 fimbriae, found in a few species of *Salmonella* and thought to be mutant CRM forms (serologically cross-reacting material) of type-I, are non-adhesive (Old & Payne, 1971). Type-1 fimbriae are protein structures found in most strains of enterobacteria (Old, 1963; Brinton, 1965). Bacteria with type-1 fimbriae adhere to fungal, plant and animal cells including erythrocytes (Duguid et al. 1955) and free fimbriae adhere by their tips to polystyrene latex particles (Brinton, 1965). The haemagglutinins, or adhesins, are most simply detected by demonstrating the clumping of erythrocytes by fimbriate bacteria. Guinea-pig, fowl and horse erythrocytes are most strongly agglutinated by the type-I fimbriae and because this interaction is inhibited by D-mannose and methyl α-D-mannoside (Duguid & Gillies, 1957), the adhesins are termed mannose-sensitive (MS). Other MS adhesins, found on the type-5 fimbriae of *Pseudomonas echinoides*, show maximum adsorption to sheep erythrocytes, a species weakly agglutinated by type-I fimbriae (Heumann & Marx, 1964). The type-6 fimbriae of *Klebsiella ozaenae* may bear yet another type of MS adhesin (Duguid et al. 1966). Mannose-resistant adhesins whose interaction with erythrocytes is not inhibited by D-mannose have been described for the type-3 fimbriae of *Klebsiella aerogenes* and the type-4 fimbriae of Proteus species (Duguid, 1959; Shedden, 1962).

Fimbriate bacteria collect at water–air interfaces either by adhesion or by flotation. For example, when subcultured in aerobic static broth, salmonella organisms bearing type-I
fimbriae form a surface pellicle that allows increased access to atmospheric oxygen and thus fimbriate bacteria grow to higher population densities in aerobic static broth than comparable non-fimbriate bacteria (Old et al. 1968; Old & Duguid, 1970, 1971). This pellicle-forming ability also is inhibited by D-mannose and methyl α-D-mannoside suggesting that it depends on the same surface property of the fimbriae as the haemagglutinating activity (Old et al. 1968).

The adhesion of type-1 fimbriae to their receptor sites on red cells has been investigated in more detail as well as the ability of monosaccharides and oligosaccharides of different configurations to inhibit this reaction. The studies were made with fimbriate cultures of *Salmonella typhimurium* and *Shigella flexneri*, two organisms whose type-1 fimbriae have closely similar adhesive affinities for different substrates, but entirely different antigenic specificities (Duguid & Campbell, 1969).

**METHODS**

*Compounds tested for inhibitory activity.* Most of the compounds tested were commercial preparations of the highest purity available. All were used without further purification, and details of sources are given in Table 3.

*Erythrocytes.* The red cells of citrated guinea-pig or horse blood were separated by centrifuging, washed with 0.85% (w/v) NaCl and made up to 3% (v/v) in 0.85% (w/v) NaCl (about 4 x 10^8 cells/ml).

*Haemagglutinating bacteria.* *Salmonella typhimurium* strain LT2 and *Shigella flexneri* type 1a, strain F111, were two strains of genotypically type-1 fimbriate bacteria from the collection of Professor J. P. Duguid. They were maintained on Dorset's egg slants at ambient temperature. The test bacterium was plated on nutrient agar (Oxoid Nutrient Agar) and incubated at 37°C for 24 h. A single colony was subcultured into nutrient broth (Oxoid Nutrient Broth no. 2) and incubated aerobically and statically at 37°C for 48 h. After three or more similar 48 h subcultures, the cultures were richly fimbriate and strongly agglutinating with guinea-pig red cells. These cultures were killed by formaldehyde (0.1%, w/v) and used as a source of haemagglutinins. Occasionally, live bacteria were tested.

*Tile haemagglutination test.* One drop (0.05 ml) of a 3% (v/v) suspension of red cells was mixed with an equal volume of the fimbriate bacterial suspension in the depressions of a porcelain tile, and mixed by rocking the tile on a mechanical shaker at room temperature. The degree of agglutination of the red cells at the end of 10 min was assessed in arbitrary units from − to +++.

*Tube haemagglutination test.* To 0.5 ml of a 1% (v/v) red cell suspension in small tubes (6.5 x 1 cm) was added an equal volume of the test bacterial suspension and the contents were mixed and left overnight at 4°C before reading. Fimbriate bacteria agglutinated the red cells; this was indicated by the formation of a loose, diffuse carpet of red cells on the bottom of the tube.

The minimum haemagglutinating dose (MHD) of bacteria from a culture was measured as the smallest number of bacteria/ml in the mixture with red cells that gave visible haemagglutination within 10 min in a tile test.

The haemagglutinating power (HP) of a culture was measured as 10^11 divided by the minimum haemagglutinating dose of bacteria (Duguid, 1959). For example, when a culture had an HP = 5000, the MHD was 2 x 10^7 bacteria/ml.

*Haemagglutination-inhibition tests.* The ability of a carbohydrate to inhibit the agglutination of guinea-pig or horse red cells by fimbriate bacteria was assessed by two methods: (i)†Tile inhibition test. Serial doubling dilutions of a 10% (w/v) solution of the carbohydrate were
Table 1. Activities of different carbohydrates in tile haemagglutination-inhibition tests of richly fimbriated cultures of Salmonella typhimurium (HP = 1100) and Shigella flexneri (HP = 1125) with guinea-pig or horse* red cells

The carbohydrate was serially diluted in a 3% suspension of red cells and one drop from each dilution mixed in the depressions of a porcelain tile with an equal volume (0·05 ml) of fimbriate bacterial suspension. Haemagglutination or inhibition of haemagglutination was read after 10 min shaking.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Minimum inhibitory concentration (% w/v) of the carbohydrate against 7·5 MHD of Salmonella typhimurium</th>
<th>Minimum inhibitory concentration (% w/v) of the carbohydrate against 3·5 MHD of Shigella flexneri</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melezitose</td>
<td>1·25 to 2·5</td>
<td>N</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>0·1</td>
<td>1·25</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>0·0078</td>
<td>0·125</td>
</tr>
<tr>
<td>Methyl α-D-mannoside</td>
<td>0·0078</td>
<td>0·62</td>
</tr>
</tbody>
</table>

* The MIC was similar with either guinea-pig or horse red cells.

N = No inhibition at the maximum tested concentration of carbohydrate (2·5%).

The final concentrations of bacteria in the test mixtures were Salmonella typhimurium 7·5 × 10⁸/ml and Shigella flexneri 3·45 × 10⁹/ml.

made with a 3% (v/v) suspension of red cells as diluent. One drop (0·05 ml) of each dilution was mixed with equal volumes of the fimbriate bacterial suspension as described above;
(ii) Tube inhibition test. Similar dilutions of the test carbohydrate were made with a 1% (v/v) red cell suspension as diluent, and 0·5 ml of each dilution mixed with an equal volume of fimbriate bacterial suspension in tubes as described above. Inhibition of the agglutination resulted in the red cells forming a small, compact button on the bottom of the tube.

In the tile and tube tests, the minimum inhibitory concentration (MIC) of the test carbohydrate was the smallest concentration of carbohydrate in the mixture with bacteria and red cells that completely prevented haemagglutination.

RESULTS AND DISCUSSION

Tile tests. All carbohydrates were screened by the tile test used previously to investigate the inhibitory effects of various carbohydrates on fimbriate Shigella flexneri cultures (Duguid & Gillies, 1957). The inhibitory effects of different concentrations of four carbohydrates, D-mannose, methyl α-D-mannoside, D-fructose and melezitose, were tested quantitatively on the haemagglutination by fimbriate cultures of Salmonella typhimurium LT2 and Shigella flexneri FIA1 of similar haemagglutinating power (HP = 1100 and 1125 respectively). At least ten times more of an inhibitory carbohydrate was required to inhibit the haemagglutination by 3·5 MHD of shigella organisms than that by 7·5 MHD of salmonella organisms (Table 1). Thus, it was difficult to detect the inhibitory effect of fructose on 3·5 MHD of the shigella whereas this carbohydrate strongly inhibited the haemagglutination given by 7·5 MHD of Salmonella typhimurium. The very weak inhibitory effect of melezitose was observed only with salmonella organisms.

A few tile tests were made with live bacteria and these showed the same pattern of inhibitions by different carbohydrates as did cultures killed by the addition of formaldehyde. Treatment with formaldehyde thus appeared not to alter either the adhesive or inhibition-sensitivity properties of the fimbriae.

Tube tests. This method of testing for haemagglutination-inhibition was rather more sensitive than the tile test, since, for example, it detected the inhibition by fructose of
Table 2. Activities of different carbohydrates in tube haemagglutination-inhibition tests of Shigella flexneri with guinea-pig red cells

The carbohydrate was diluted in a 1% suspension of guinea-pig red cells. 0.5 ml of each dilution was mixed with an equal volume of fimbriate bacterial suspension. The end-point of inhibition of the fimbrial haemagglutination was read after overnight incubation at 4°C.

<table>
<thead>
<tr>
<th>No. of MHD of bacteria in test</th>
<th>D-Mannose</th>
<th>Methyl α-D-mannoside</th>
<th>1,5-Anhydro-D-mannitol</th>
<th>D-Manno-heptulose</th>
<th>2-Deoxy-D-glucose</th>
<th>L-Mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.025</td>
<td>0.025</td>
<td>0.013</td>
<td>0.016</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>0.013</td>
<td>0.013</td>
<td>0.003</td>
<td>0.004</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>7.5</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>—</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>3.8</td>
<td>0.0016</td>
<td>0.0016</td>
<td>0.0016</td>
<td>—</td>
<td>2.5</td>
<td>N</td>
</tr>
<tr>
<td>1.9</td>
<td>0.0016</td>
<td>0.0016</td>
<td>0.0016</td>
<td>—</td>
<td>—</td>
<td>N</td>
</tr>
</tbody>
</table>

N = No inhibition at the maximum tested concentration of carbohydrate (2.5%); — = not tested.

haemagglutination by Shigella flexneri, which was not detected by the tile test. All the remaining results are given for tube tests but do not conflict with those for the tile tests except in the direction of greater sensitivity. It was important to estimate the inhibitory potency of different carbohydrates as far as possible on a single culture of known HP because the MIC of a carbohydrate was influenced by the HP of the culture and by the number of MHD used in the test. For example, the carbohydrates in Table 2 were tested as inhibitors of the haemagglutination reaction of a fimbriate culture of S. flexneri F111 (HP = 5000, 1.2 x 10^8 bacteria/ml) in test systems containing between 1.9 and 30 MHD of fimbriate bacteria. The relationship between the MIC of the carbohydrate and the number of bacteria tested in the haemagglutination test was only very approximately linear, perhaps due to a lack of sensitivity in the test system. Some substances at 2.5% in systems containing only a very few MHD of bacteria, seemed to be inhibitory though they were not when tested against a larger number of MHD of bacteria. However, the assignment of a substance as an inhibitor from its effect in tests with low numbers of MHD was difficult because the haemagglutination test itself is of low sensitivity under such conditions. For this reason, most experiments were made with at least 5 to 30 MHD of fimbriate bacteria from cultures with HP in the range 1000 to 5000.

Monosaccharides and oligosaccharides. D-Mannose and D-fructose were the only monosaccharides originally screened that significantly inhibited the activity of the fimbrial adhesins of type-1 fimbriae. D-Fructose was less inhibitory than D-mannose and in tube tests with 30 MHD of fimbriate Shigella flexneri and guinea-pig red cells four times more D-fructose (0.078%, w/v, or 43 μmol/ml) than D-mannose (0.02%, w/v, or 1.1 μmol/ml) was needed to give complete inhibition of haemagglutination. Of the oligosaccharides tested, only melezitose had any inhibitory activity, and this trisaccharide (α-D-glucopyranosyl-β-D-fructofuranosyl-α-D-glucopyranose) was a poor inhibitor, being active only at very high concentrations and more effectively inhibiting the haemagglutinin of Salmonella typhimurium than that of Shigella flexneri. It consistently inhibited 7.5 MHD of fimbriate cultures of salmonella at concentrations of 1.25 to 2.5% (12 to 24 μmol/ml). No inhibition was seen with fimbriate cultures of shigella except when these were examined in tests containing fewer than 5 MHD of bacteria. This finding confirmed the greater sensitivity to inhibition shown by the salmonella organisms than the shigella organisms in the tile tests. L-Mannose was not inhibitory. None of the sugar alcohols, the tetroseS, pentoses or their derivatives was inhibitory nor was any of the other hexose sugars listed in Table 3.
Inhibition of fimbrial haemagglutination

Table 3. Activity of various carbohydrates (and source*) in inhibiting fimbrial haemagglutination

All compounds were initially screened at a concentration of 2.5% (w/v) in a tile or tube test with guinea-pig or horse red cells. Any substance inhibiting at 2.5% was retested by the tube test to determine its MIC.†

**Strongly inhibitory at 0.001 to 0.02 %, w/v**

<table>
<thead>
<tr>
<th>Mannose and derivatives</th>
<th>Mannose and derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Mannose (A, C)</td>
<td>d-Mannheptulose (B)</td>
</tr>
<tr>
<td>Methyl α-d-mannoside (E)</td>
<td>α-d-Mannose-1-phosphate (F)</td>
</tr>
<tr>
<td>1,5-Anhydromannitol (G)</td>
<td></td>
</tr>
</tbody>
</table>

**Modestly inhibitory at 0.05 to 0.2 %, w/v**

<table>
<thead>
<tr>
<th>Yeast mannan (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Fructose (A, C, D, E)</td>
</tr>
</tbody>
</table>

**Weakly inhibitory at 0.75 to 2.5 %, w/v**

<table>
<thead>
<tr>
<th>Mannose and derivatives</th>
<th>Mannose and derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Deoxy-d-glucose (E)</td>
<td>Methyl β-d-mannopyranoside (G)</td>
</tr>
<tr>
<td>6-Deoxy-d-mannose (H)</td>
<td>Methyl 1-thio-β-d-mannopyranoside (G)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Mannosamine (F)</td>
</tr>
<tr>
<td>N-Acetyl-d-mannosamine (F)</td>
</tr>
<tr>
<td>d-Mannose-5-phosphate (F)</td>
</tr>
<tr>
<td>α-Mannohexitol (F)</td>
</tr>
</tbody>
</table>

**Non-inhibitory at 2.5 %, w/v**

<table>
<thead>
<tr>
<th>Mannose and derivatives</th>
<th>Mannose and derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Mannose (B)</td>
<td>Methyl 2,3-anhydro-α-d-mannoside (G)</td>
</tr>
<tr>
<td>d-Mannosamine (F)</td>
<td>2,3-di-O-methyl d-mannose (G)</td>
</tr>
<tr>
<td>N-Acetyl-d-mannosamine (F)</td>
<td>Mannobiase (G)</td>
</tr>
<tr>
<td>d-Mannose-6-phosphate (F)</td>
<td>Mannotriose (G)</td>
</tr>
<tr>
<td>α-Mannohexitol (F)</td>
<td>d-Mannitol (C)</td>
</tr>
</tbody>
</table>

**Tetrose**

<table>
<thead>
<tr>
<th>Pentoses and derivatives</th>
<th>Hexoses and derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Erythrose (A)</td>
<td>2-Deoxy-d-ribose (E)</td>
</tr>
<tr>
<td>d-Arabinose (A)</td>
<td>d-Xylose (A)</td>
</tr>
<tr>
<td>d-Lyxose (E)</td>
<td>d-Glucose (A)</td>
</tr>
<tr>
<td>d-Ribose (A)</td>
<td>d-Galactose (A)</td>
</tr>
</tbody>
</table>

**Hexoses and derivatives**

<table>
<thead>
<tr>
<th>Sugar alcohols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adonitol (C)</td>
</tr>
<tr>
<td>Dulcitol (A)</td>
</tr>
</tbody>
</table>

**Oligosaccharides**

<table>
<thead>
<tr>
<th>Celllobiose (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen (E)</td>
</tr>
<tr>
<td>Inulin (E)</td>
</tr>
<tr>
<td>Lactose (A)</td>
</tr>
<tr>
<td>Maltose (A)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Melibiose (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raftinose (A)</td>
</tr>
<tr>
<td>Starch (A)</td>
</tr>
<tr>
<td>Sucrose (A)</td>
</tr>
<tr>
<td>Trehalose (A)</td>
</tr>
</tbody>
</table>

**Miscellaneous**

<table>
<thead>
<tr>
<th>DL-Glyceraldehyde (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol (A)</td>
</tr>
<tr>
<td>Meso-inositol (A)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amygdalin (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicin (A)</td>
</tr>
</tbody>
</table>


† The range of MIC values quoted are those estimated in tests containing 7.5 to 8 MHD of Shigella flexneri.

‡ No accurate MIC available.
Derivatives of mannose. Table 3 summarizes the findings for the whole series of compounds including several derivatives of D-mannose. D-Mannose was about as strongly inhibitory as any carbohydrate tested, and equally effective were derivatives of D-mannose substituted at C-1 (methyl α-D-mannoside, D-mannoheptulose, α-D-mannose-1-phosphate and 1,5-anhydromannitol). When tested (in 1 ml total volume) against 30 MHD of fimbriate shigella organisms, the following amounts were inhibitory: D-mannose (1.39 μmol), methyl α-D-mannoside (1.13 μmol), 1,5-anhydromannitol (0.8 μmol) and D-mannoheptulose (1.46 μmol). Presumably the pyranose ring form of D-mannose was active in this reaction with fimbriae because the open chain D-mannitol and α-mannoheptitol, derived from D-mannose and D-mannoheptulose, were non-inhibitors, and so too was methyl α-D-mannofuranose.

The fact that the C-1 substituted derivatives of mannose were active suggested that the C-1 hydroxyl group played no essential role in the fimbrial inhibition.

Unmodified hydroxyl groups at C-2, C-3, C-4 and C-6 of the D-mannose molecule seemed to be required for maximum binding to the fimbrial protein. For example, D-glucose, D-altrose and D-talose, the C-2, C-3 and C-4 epimers of D-mannose, at 2.5% (139 μmol/ml) failed to inhibit even very low numbers of MHD of either Shigella flexneri or Salmonella typhimurium. Further confirmation came from the findings that the substituted derivatives of D-mannose (2,3-di-O-methyl D-mannose and 2,3-anhydro-α-D-mannoside) failed to inhibit the reaction. The importance of the C-2 hydroxyl group in the inhibitory D-mannose molecule was apparent because both D-mannosamine and N-acetyl-D-mannosamine were non-inhibitors. The participation of the C-2 hydroxyl group was also apparent from the observation that 2-deoxy-D-glucose inhibited the reaction slightly, when tested at the high concentration of 2.5% (152 μmol/ml) in systems containing only a very few MHD of bacteria.

Modification of the C-6 hydroxyl caused a great loss in activity. 6-Deoxy-D-mannose (D-rhamnose, 0.88%, w/v, 54 μmol/ml) inhibited the haemagglutination by 8 MHD of fimbriate shigella organisms, but D-mannose-6-phosphate was not inhibitory at any concentration tested up to 2.5%. It might be that the phosphate group at position C-6 in mannose-6-phosphate introduced considerably more steric disturbance in the molecule than the methyl group of 6-deoxy-D-mannose.

There seems to be a specific requirement for the α-configuration at the C-1 position in the inhibitory molecule, because, whereas mannose-containing carbohydrates that retained their α-linkage were still good inhibitors (e.g. methyl α-D-mannoside), carbohydrates with a β-configuration (e.g. methyl β-D-mannoside and methyl α-thio-β-D-mannopyranose) were poor inhibitors or non-inhibitors. These two β-substituted carbohydrates, tested in parallel with 1,5-anhydromannitol, inhibited the haemagglutination by 4 MHD of fimbriate Salmonella typhimurium only at a greater concentration (0.01%, w/v) than 1,5-anhydromannitol which was active at 0.001%. Only small amounts of these carbohydrates were available and so replicate tests were not possible. The specific requirement for the α-linkage was confirmed by the failure of the β-(1→4)-linked mannobiose (4-O-β-D-mannopyranosyl-D-mannose) and mannotriose to inhibit haemagglutination, even when tested at concentrations as high as 2.5% (w/v). Yeast mannan, a commercial preparation of unknown structure, was effective in inhibiting the haemagglutination by 8 MHD of Shigella flexneri at a concentration of 0.156% (w/v) in experiments in which D-mannose, methyl α-D-mannoside and D-fructose inhibited at 1.11, 1.03 and 4.3 μmol/ml respectively. This demonstration that yeast mannan was an inhibitor of moderate activity suggested that this commercial preparation was an α-linked mannose polymer, which would be in agreement with the observations of Jones & Ballou (1968) on several preparations of yeast mannan.
Inhibition of fimbrial haemagglutination

No inhibition of the fimbrial reaction was observed with D-lyxose, D-xylitol, L-arabinose, D-glucose, D-galactose, L-rhamnose, lactose, maltose, sucrose, D-glucitol, mannitol, inositol, even at concentrations between 0-5 and 2-5 % (w/v). My finding that the type-1 fimbriae of *Salmonella typhimurium* had a pattern of inhibition sensitivity to different carbohydrates closely similar to that of the type-1 fimbriae of *Escherichia coli* and *Shigella flexneri* is interesting because of the complete antigenic difference between the fimbriae of salmonellas and those of *E. coli* and shigellas (Duguid & Campbell, 1969). Despite the arbitrary definition of an inhibitor used in this paper, namely a substance that completely inhibited the fimbriae-erythrocyte interaction at a concentration not higher than 2-5 % (w/v), very few naturally occurring sugars were inhibitors. The two most effective monosaccharide inhibitors were D-mannose and D-fructose. Several commercial preparations of D-fructose were tested, all of which caused inhibition. Paper chromatography showed them to be pure preparations uncontaminated with D-mannose so that their effect seemed real. There is one form of D-fructose, \( \beta \)-D-fructopyranose, in which the stereochemical configuration required in the inhibitory D-mannose molecule is almost reproduced, the hydroxyl groups at C-2, C-3, C-4 and C-6 of D-mannose being matched by the corresponding hydroxyl groups at C-1, C-3, C-4 and C-5 of D-fructose. However, the present experimental data are insufficient to eliminate the possibility that D-fructose is an inhibitor by a mechanism that has nothing to do with its structural similarity to D-mannose, i.e. there may be sites at which D-fructose attaches to the fimbrial molecule other than the mannose-adsorbing sites. Although melezitose was a poor inhibitor, its effect could be consistently reproduced for both shigella and salmonella adhesins especially when the number of haemagglutinating doses tested was low. There is no obvious relationship between melezitose and mannose. This trisaccharide contains two \( \alpha \)-D-glucopyranosyl residues and a D-fructofuranosyl residue; why this combination of sugars should work is not clear. Its activity again suggests that there may be more than one site of inhibition on the fimbriae. Melezitose was the only inhibitor among the many di- and tri-saccharides examined.

Observations of the inhibition of bacterial haemagglutination by D-mannose and methyl \( \alpha \)-D-mannoside were made by Collier and his colleagues with the haemagglutinin of *Escherichia coli* (Collier & de Miranda, 1955; Collier & Jacob, 1955; Collier, Tiggelman-van Krugten & Tjong A Hung, 1955). This haemagglutinin, which was probably fimbrial in nature, was inhibited not only by D-mannose and methyl \( \alpha \)-D-mannoside but also by D-glucose, L-sorbose, sucrose and trehalose, especially when small numbers of agglutinating doses were tested (Collier & Tiggelman-van Krugten, 1957). Because type-1 fimbrial haemagglutginins of *Shigella flexneri* and *E. coli* might have been slightly different in activity, despite their serological relatedness, I tested fimbriate cultures of both species in the range from 2 to 16 MHD, but was unable to demonstrate inhibition of either species by these four sugars.

There is a remarkable similarity in the inhibition pattern of the fimbrial adhesin-erythrocyte system and the concanavalin-dextran system (Goldstein, Hollerman & Smith, 1965; Smith & Goldstein, 1967; So & Goldstein, 1968). Concanavalin-A, a protein haemagglutinin isolated from jack bean meal, agglutinates the erythrocytes of the cat, dog, guinea-pig and rabbit but not those of the cow, goat, horse, man, pig or sheep. The haemagglutinin of type-1 fimbriae shows an almost similar agglutination specificity, the most obvious difference being that it reacts strongly with horse and pig red cells. Goldstein and his co-workers, in a series of elegant studies, investigated some of the requirement sites for inhibition of this phytohaemagglutinin and showed that unmodified hydroxyl groups at C-3, C-4 and C-6 positions of D-glucopyranose and D-mannopyranose were essential for binding to the protein, and that substitution of the C-2 hydroxyl group was tolerated more in D-glucose than D-mannose.
They showed, too, that α-linked sugars were more potent inhibitors than the corresponding β-linked derivatives, and from their work with mannans from various micro-organisms, suggested that the combining sites were extensive, possibly complementary to a sequence of several α-(1→2)-linked β-mannopyranosyl residues. However, whereas the concanavalin-A-dextran system was inhibited by the monosaccharides, D-mannose, D-fructose, D-glucose and L-sorbose in that order of activity, inhibition of fimbrial haemagglutination by D-glucose or L-sorbose could not be demonstrated, even when these were included in the test system at 140 μmol/ml. The inability of these sugars to inhibit fimbrial adhesiveness had been demonstrated previously; unlike D-mannose and methyl α-D-mannoside, L-sorbose and D-glucose (0.2%, w/v) in broth neither delayed the time of appearance of fimbrial pellicles of fimbriate strains of Salmonella (Old et al. 1968) nor prevented the successful outgrowth of small numbers of fimbriate bacteria grown in mixed culture with large numbers of non-fimbriate bacteria (Old & Duguid, 1970). Other slight differences in the two systems are apparent because maltose, trehalose and sucrose, inhibitors of the concanavalin-A system, did not inhibit the type-1 fimbrial haemagglutinin, and because melezitose was as potent as methyl α-D-mannoside as an inhibitor of the concanavalin-A system. Nevertheless, the similarities between the two haemagglutinins—concanavalin-A and fimbriae—are strong. It is suggested that whereas these haemagglutinating proteins may differ in configuration to some extent, their receptors in the red cells may be quite similar.

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REFERENCES


Inhibition of fimbrial haemagglutination


Smith, E. E. & Goldstein, I. J. (1967). Protein-carbohydrate interaction. V. Further inhibition studies directed toward defining the stereochemical requirements of the reaction sites of concanavalin A. Archives of Biochemistry and Biophysics 121, 88–95.