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CARBOHYDRATE SPECIFICITY OF THE SURFACE LECTINS OF *Escherichia coli*, *Klebsiella pneumoniae*, AND *Salmonella typhimurium*[*,†]

NURIT FIRON[‡], ITZHAK OFEK[§], AND NATHAN SHARON[‡]

Department of Biophysics, The Weizmann Institute of Science, Rehovoth, and Department of Human Microbiology, The Sackler School of Medicine, Tel-Aviv University, Tel-Aviv (Israel)

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ABSTRACT

A large number of linear and branched oligosaccharides and several glycosides of D-mannose were tested for their inhibitory activity on the agglutination of yeast cells or guinea pig erythrocytes by three D-mannose-specific enteric bacteria possessing type 1 fimbriae. With *Escherichia coli* 346, the best inhibitors found are the α glycosides of the branched oligosaccharides α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-α-D-Man*p*-(1→6)-α-D-Man*p*-(1→3)-D-Man*p* and α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-α-D-Man*p*-(1→6)-[α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3)]-D-Man*p* and the trisaccharide α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-D-GlcNAc, all of which are 21–30 times more inhibitory than methyl α-D-mannopyranoside. The aromatic glycoside *p*-nitrophenyl α-D-mannopyranoside was also a strong inhibitor (30 times more inhibitory than methyl α-D-mannopyranoside), whereas the corresponding β-D-glycoside was only a weak inhibitor (approximately as methyl α-D-mannopyranoside). A nearly identical pattern of inhibitory activity was observed with the fimbriae. This suggests that the combining site of the *E. coli* fimbrial lectin is in the form of an extended pocket on the surface of the lectin corresponding to the size of a trisaccharide and fitting best the structure α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-D-GlcNAc. Since *p*-nitrophenyl α-D-mannopyranoside is a strong inhibitor, the existence of a hydrophobic region in the combining site or close to it was assumed. The combining site of the *Klebsiella pneumoniae* fimbrial lectin is probably similar to that of *E. coli*, but that of the *Salmonella typhimurium* fimbrial lectin differs considerably. It appears that the combining sites of the three bacterial lectins tested exhibit preference for structures found in *N*-glycosylic oligomannoside units of mammalian cell surface glycoproteins.

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‡The Weizmann Institute of Science.

§Tel-Aviv University

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INTRODUCTION

Bacterial adherence to surfaces of animal tissues has recently gained increasing attention as an important initial event in the pathogenesis of bacterial infection[1,2]. The adherence of many strains of *Escherichia coli* and other enteric bacteria to epithelial cells, as well as the agglutination of guinea pig erythrocytes or D-mannan-containing yeast cells (*e.g., Saccharomyces cerevisiae* and *Candida albicans*) by these bacteria, is inhibited specifically by small concentrations of D-mannose (**1**), methyl α-D-mannopyranoside (**5**) and yeast D-mannan[3,4]. Both the D-mannose-specific (MS) adherence and the MS agglutination are mediated by bacterial-surface structures in the form of type 1 fimbriae (or pili). These fimbriae are[5,6] thin (7 nm in diameter) and long (~50–250 nm) appendages consisting of protein subunits known as pilin, molecular weight 17000. Isolated fimbriae agglutinate guinea pig erythrocytes or yeast cells, and this agglutination is also specifically inhibited by low concentrations of D-mannose (**1**) or methyl α-D-mannopyranoside (**5**)[7–10]. Thus, the fimbriae exhibit properties characteristic of lectins[11].

Until recently, the specificity of the MS bacterial lectins had been examined only with a limited variety of sugars, mostly simple monosaccharides[3,4,12]. Perhaps the only exception is the study of Old[13], who tested the effect of various monosaccharides, as well as several simple oligosaccharides, on the MS agglutination of guinea pig and horse erythrocytes by certain strains of *Salmonella typhimurium* and *Shigella flexneri* possessing type 1 fimbriae. Modification of OH-2, -3, -4, or -6 of the D-mannopyranosyl residue resulted in loss of inhibitory activity, showing that these groups are required for binding to the bacteria. The α configuration of the D-mannose unit was also found to be important, since carbohydrates containing a β-linked D-mannose unit were much poorer inhibitors than those containing the α-linked unit. Of the oligosaccharides examined by Old[13], none proved to be an effective inhibitor. It should be pointed out that the data on the relative inhibitory activity of the various sugars tested in the earlier studies were only semiquantitative, owing to a lack of accurate methods for measuring agglutination.

Quantitative studies of the specificity of the bacterial-surface lectins are of interest for several reasons: (a) They permit better characterization of the structures of the combining sites of these lectins; (b) they give an insight into the nature of the interaction between the bacteria and cell surfaces; and (c) they may provide information for the design of more effective inhibitors for the prevention of adhesion to mucosal surfaces *in vivo*[14], and perhaps also of natural infection.

We have recently demonstrated that the specificity of the *E. coli* fimbrial lectin can be studied quantitatively by examining the relative inhibitory activity of various D-mannose-containing glycosides and oligosaccharides on the rate of agglutination of yeast cells by the bacteria, as measured in an aggregometer. A linear correlation was found between the percent of inhibition of yeast aggregation and the logarithm of inhibitor concentration, permitting the determination of its relative inhibitory activity. Preliminary data on these findings have been re-

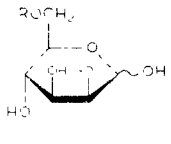
ported[15,16]. We have now extended these studies by use of additional sugars, as well as two other MS bacteria, *Klebsiella pneumoniae* and *S. typhimurium*, and have also used guinea pig erythrocytes in the agglutination experiments.

EXPERIMENTAL

Materials. — Methyl α-D-mannopyranoside (**5**), D-mannose (**1**), L-mannose (**3**), and 2-deoxy-D-*arabino*-hexose (**4**) were purchased from Pfanstiehl Laboratories Inc. (Waukegan, IL 60085, U.S.A.); *p*-nitrophenyl α-D-mannopyranoside (**6**) from Koch–Light Laboratories Ltd. (Colnbrook, Berkshire SL3 0BZ, U.K.); and *p*-nitrophenyl β-D-mannopyranoside (**8**), α-D-mannopyranosyl phosphate (**7**), methyl α-D-glucopyranoside (**9**), and D-mannose 6-phosphate (**2**) from Sigma Chemical Co. (St. Louis, MO 63178, U.S.A.). The oligosaccharides used were generous gifts of several investigators: **10–12** isolated from yeast mannan (T. Nakajima, Tohoku University, Sendai, Japan); **13** also from yeast mannan (C. E. Ballou, University of California, Berkeley, California); synthetic **14–16** (H. Paulsen, University of Hamburg, West Germany); **15, 17,** and **18** isolated from the urine of patients with mannosidosis (A. Lundblad, University of Lund, Sweden); synthetic branched oligosaccharides **19–23** (T. Ogawa, Institute of Physical and Chemical Research, Saitama, Japan); **24–26** from the urine of patients with GM1 gangliosidosis (J. Montreuil and G. Strecker, University of Lille, France); and synthetic **27–29** (J. Lönngren, Stockholm University, Sweden). All other chemicals were of analytical grade, obtained from commercial sources.

Cultivation of bacteria. — *E. coli* strain 346 and *K. pneumoniae* were isolated from patients with urinary tract infection, and *S. typhimurium* was from the collection of the Department of Human Microbiology (Tel-Aviv University). To obtain bacteria enriched in type 1 fimbriae and expressing high lectin-activity, the serial passage technique was used[3,17]. For this purpose, the bacteria were serially passaged in test tubes containing 5 mL of nutrient broth (Difco), incubated at 37° for 48 h under static conditions. After each passage, the bacteria were harvested by centrifugation, washed once with phosphate buffered saline (PBS; 20mM phosphate in 0.15M sodium chloride, pH 7.4), and assayed for their yeast agglutinating activity as described later. Passaging was continued until 10^7 cells/mL gave a rate of aggregation of 15–20 units/min. At this point, the bacteria were grown once in 500 mL of broth under the aforementioned conditions. The organisms were harvested by centrifugation, washed three times with PBS, and resuspended in the same buffer. The bacterial concentration was adjusted to Abs. 0.5 (O.D.) (Junior Coleman, 610 nm). For most of the experiments, the cells were kept frozen at −70° until used.

Preparation of E. coli *fimbriae.* — Type 1 fimbriae were isolated from *E. coli* 346 by mechanical agitation of the bacteria according to Eshdat *et al.*[6] with four consecutive precipitations in 0.1M magnesium chloride at 4°. The last precipitate was suspended in 5mM Tris · HCl buffer (pH 8.0, containing 0.02% azide) and stored at 4° until used.



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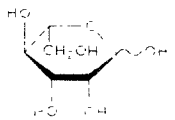
2 R = H PO



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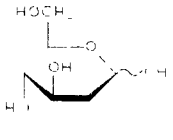

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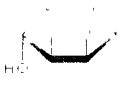
6 R = $C_8H_{13}NO_5$
7 R = Et



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9

α-D-Manp-(1→2)-D-Man

10

α-D-Manp-(1→3)-D-Man

11

α-D-Manp-(1→6)-D-Man

12

α-D-Manp-(1→2)-α-D-Manp-(1→2)-D-Man

13

β-D-Manp-(1→4)-D-GlcNAc

14

α-D-Manp-(1→3)-β-D-Manp-(1→4)-D-GlcNAc

15

α-D-Manp-(1→6)-β-D-Manp-(1→4)-D-GlcNAc

16

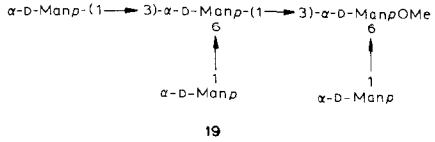
α-D-Manp-(1→2)-α-D-Manp-(1→2)-α-D-Manp-(1→3)-β-D-Manp-(1→4)-D-GlcNAc

17

α-D-Manp-(1→2)-α-D-Manp-(1→2)-α-D-Manp-(1→3)-β-D-Manp-(1→4)-D-GlcNAc

18

Assay of yeast aggregation. — Aggregation of yeast cells (*S. cerevisiae*, commercial baker's yeast, a product of Fleischmann) was monitored in a Payton aggregometer, as described by Ofek and Beachey[18]. The yeast cells were suspended in PBS (0.5 mg, dry weight/mL); for each assay, 0.5 mL of the suspension was used. The bacteria were washed once in PBS before use, and diluted in PBS so that, upon addition of 10 μL of the bacterial suspension to the yeast cells, the steepest slope of the curve recorded by the aggregometer was 15–20 units/min with *E. coli* 346 or with *S. typhimurium*, 4–7 units/min with the isolated fimbriae, and 8–16 units/min with *K. pneumoniae*. The concentration of bacteria was estimated turbidimetrically with a Klett colorimeter, filter No. 66; 5×10^8 cells/mL gave a reading of 100 Klett units.



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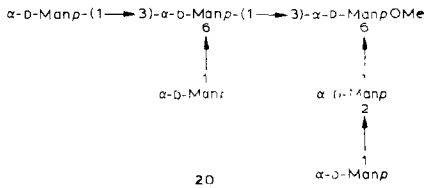

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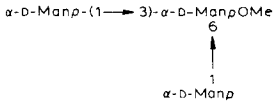

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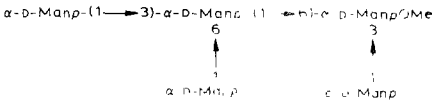

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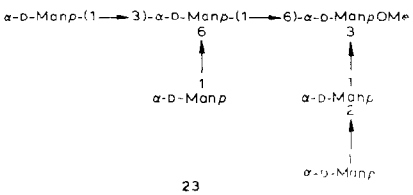

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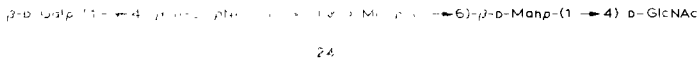

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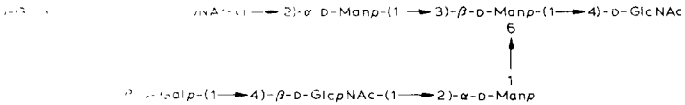

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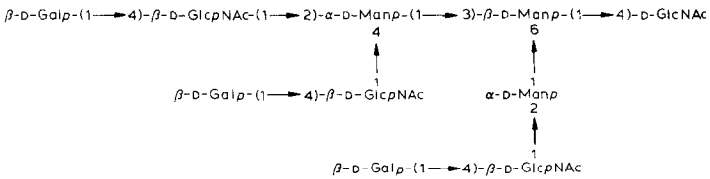

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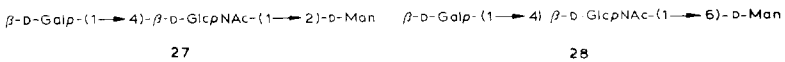

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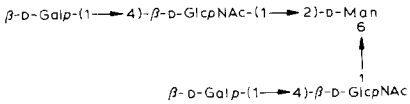

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The inhibitory sugars were routinely added in PBS (10–50 μL) to the yeast-cell suspension, followed by addition of the bacteria or fimbriae in 10 μL. The concentration of the sugars was determined by the phenol–sulfuric acid method[19] with D-mannose as standard. The percent inhibition by the sugar was calculated as 100[1 − (steepest slope with inhibitor)/(steepest slope without inhibitor)]. The percent inhibition for the various sugars was plotted as a logarithmic function of their concentration. In all cases, a highly significant linear correlation was obtained ($p < 0.05$). For each sugar, the concentration causing 50% inhibition was derived from its inhibition line, and the relative inhibitory activity in comparison to that of **5** was calculated.

Agglutination of guinea pig erythrocytes. — Agglutination of guinea pig erythrocytes (freshly drawn) was monitored in the aggregometer as described above for yeast aggregation. The erythrocytes were suspended in PBS, and their concentration was adjusted to give a reading of 30% transmission in the aggregometer. The bacteria were diluted in PBS so that, in the absence of inhibitor, the steepest slope recorded by the aggregometer for hemagglutination by *E. coli* 346 and by *S. typhimurium* was 6–9 and 3 units/min, respectively. The inhibitory sugars were added in 10–50 μL of PBS, and the percent inhibition was calculated as described earlier.

RESULTS

The rate of yeast aggregation by two bacterial strains, as monitored in the aggregometer in the absence and presence of inhibitory sugars, is shown in Fig. 1. As can be seen, addition of D-mannose derivatives caused a decrease in the aggregation rate. It is also apparent that **6** is a much stronger inhibitor of the agglutination of yeast cells by *E. coli* than is **5**, whereas both compounds are almost equally inhibitory with *S. typhimurium*.

Reproducibility of the method. — Determinations of the concentration of **5** needed to cause 50% inhibition of yeast aggregation by *E. coli*, in repetitive experiments employing the same bacterial culture, are highly reproducible (±10%), although this concentration differs from one culture to another, probably because of variations in the expression of the MS lectin on the bacterial-cell surface[18]. Thus, in three separate experiments with three different bacterial cultures of *E. coli*, the absolute concentration of **5** needed to cause 50% inhibition of yeast aggregation was 0.15, 0.17, and 0.40mM, whereas the ratio between the inhibitory activities of **6** and **5** remained in the same range, namely 30, 25, and 40, respectively (average 31 ±7.0). Excess of a noninhibitory sugar (methyl α-D-glucopyranoside, **9**) in the reaction mixtures, or of bacteria of the same strain devoid of MS activity, did not influence the 50% inhibition point of **5** (data not shown), thus excluding the possibility of nonspecific absorption of sugars by the bacteria. The slope of the inhibition curve, but not the 50%-inhibition point, decreased as the bacterial concentration

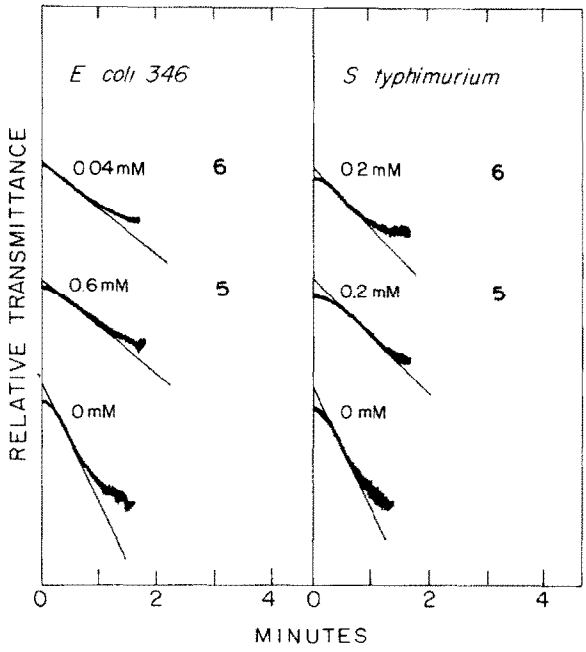

Fig. 1. Effect of methyl α-D-mannopyranoside (**5**) and *p*-nitrophenyl α-D-mannopyranoside (**6**) on the aggregation of D-mannan-containing yeast cells by *E. coli* 346 and *S. typhimurium* as measured in a Payton aggregometer.

TABLE I

EFFECT OF BACTERIAL CONCENTRATION ON THE INHIBITORY ACTIVITY BY METHYL α-D-MANNOPYRANOSIDE (**5**) OF YEAST AGGREGATION

Bacterial concentration in the reaction mixture (cells/mL)	*Slope of inhibition line*[a]	*Conc. (mM) of* **5** *causing 50% inhibition*
E. coli 346		
1.6×10^7	68	0.45
6.3×10^7	52	0.45
5.0×10^8	28	0.40
S. typhimurium		
0.1×10^8	56	0.12
2.7×10^8	49	0.16
5.4×10^8	45	0.17

[a] $\Delta\%/\Delta$ log(conc, mM).

increased (Table I). No detectable differences were observed between the 50%-inhibition point obtained by preincubation of **5** with the bacteria and those obtained by adding the sugar to the yeast suspension just before adding the bacteria (data not shown).

Inhibition of yeast aggregation by various sugars. — E. coli. The inhibition lines of various sugars for yeast agglutination by *E. coli* are shown in Fig. 2a. It should be noted that, although most of the sugars gave parallel lines of inhibition, with some the slope of the inhibition lines were considerably different. The relative inhibitory activities of the various sugars are presented in Table II. The Table also contains data for sugars for which inhibition lines are not shown (see footnote to Table II).

The various sugars can be classified into three groups according to their relative inhibitory activity. One group, comprised of compounds **1, 8, 10–14, 16–18, 24, 25**, is approximately as inhibitory as **5**. Another group includes compounds **19** and **20** that are 3–5 times more inhibitory than **5**. A third group includes the most potent inhibitors, **6, 15, 21–23**, being up to 30 times more inhibitory than 5.

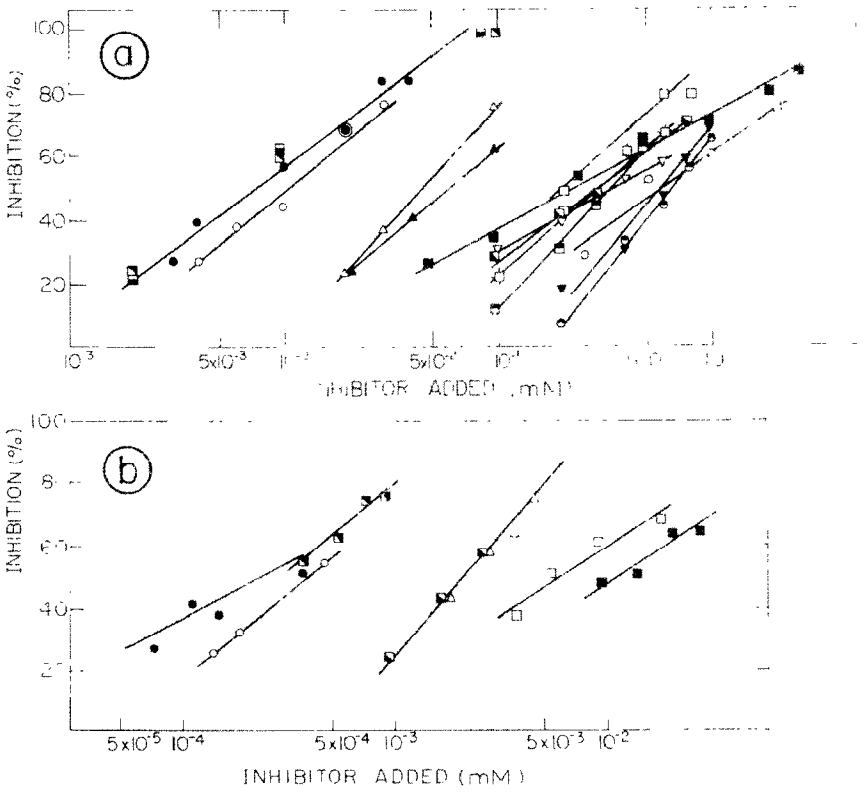

Fig. 2. Inhibition by D-mannose derivatives of yeast aggregation by *E. coli* 346 (a) and *E. coli* 346 isolated fimbriae (b). Symbols as in Table II.

TABLE II

RELATIVE INHIBITION BY D-MANNOSE DERIVATIVES OF YEAST AGGLUTINATION BY *E. coli* 346, *E. coli* FIMBRIAE, *K. pneumoniae*, AND *S. typhimurium*

Compound	*Symbol*	E. coli *346*	E. coli *isolated fimbriae*	K. pneumoniae	S. typhimurium
1	⌀	0.8	[c]	0.6	1.4
5[a]	■	1.00	1.00	1.00	1.00
6	●	30	48	16	0.4
8	▽	1.2	[c]	0.5	<0.12
10	□	1.3	1.8	5.4	0.8
11[b]		1.2	[c]	[c]	<0.2
12	▭	0.5	[c]	1.6	1.2
13	⌖	1.4	[c]	[c]	[c]
14[b]		<0.4	[c]	[c]	[c]
15	○	21	30	8	0.1
16	▼	0.7	[c]	[c]	0.75
17	◪	0.7	5.5	2.6	<0.12
18[b]		0.7	[c]	[c]	0.08
19	▲	3.5	[c]	[c]	[c]
20	△	4.7	4.8	[c]	4.0
21[b]	☆	10.5	[c]	[c]	1.2
22	▬	30	[c]	18	[c]
23	◥	30	36	[c]	2.0
24[b]		0.25	[c]	[c]	[c]
25	◓	0.6	[c]	[c]	<0.4

[a]The inhibitory activities of the D-mannose derivatives are compared relative to compound **5**, which was arbitrarily set to 1.00. Concentrations of **5** at which 50% inhibition is achieved are 0.012mM for *E. coli* 346 isolated fimbriae, 2.7mM for *K. pneumoniae*, and 0.12mM for *S. typhimurium*. In the case of *E. coli* 346, two batches of bacteria were used; **1**, **8**, **13**, **14**, **16**, **24**, and **25** were tested with one batch which required 0.45mM of **5** for 50% inhibition, and the remaining compounds were tested with the other batch which required 0.15mM of **5**. [b]Because of shortage of material or low solubility, only two inhibitory concentrations were tested, and no lines are given in Figs. 2 and 3. [c]Not tested.

TABLE III

MAXIMAL CONCENTRATIONS OF D-MANNOSE DERIVATIVES SHOWING WEAK OR NO INHIBITION OF YEAST AGGREGATION BY *E. coli* 346 AND BY *S. typhimurium*

Compound	*Maximal conc. tested (mM)*	*% Inhibition*	
		E. coli *346*	S. typhimurium
2	2.0	0	5
3	5.0	11	6
4	5.0	19	8
7	2.0	0	10
26	1.0	16	21
27	1.6	0	[a]
28	1.6	0	[a]
29	1.6	0	[a]

[a]Not tested.

TABLE IV

INHIBITORY ACTIVITY OF D-MANNOSE DERIVATIVES ON AGGLUTINATION OF GUINEA PIG ERYTHROCYTES BY *E. coli* 346 AND *S. typhimurium*

Compound	*Conc. (μM) causing 50% inhibition*		*Relative inhibitory activity*	
	E. coli *346*	S. typhimurium	E. coli *346*	S. typhimurium
5	150	40	1	1
6	1.2	60	125	0.7
8	150	[a]	1	[a]
15	3	[a]	50	[a]

[a] Not tested

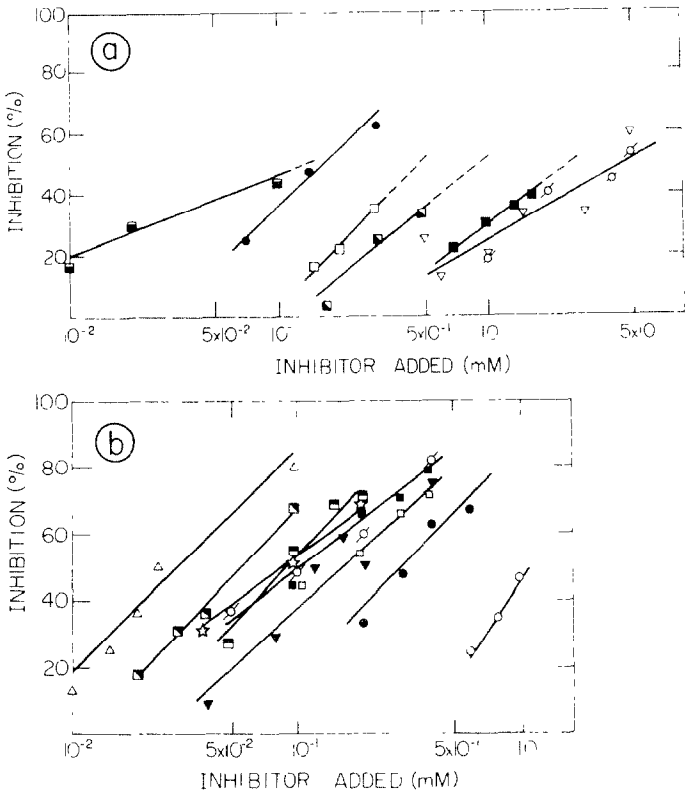

Fig. 3. Inhibition by D-mannose derivatives of yeast aggregation by *K. pneumoniae* (a) and *S. typhimurium* (b). Symbols as in Table II.

When the assays were carried out with the *E. coli* fimbriae, instead of the intact bacteria, essentially the same pattern of inhibitory activity of the sugars tested was observed (Fig. 2b). An exception was **17** which was a moderate inhibitor of yeast agglutination by isolated fimbriae, but only weakly inhibitory for yeast agglutination by the intact bacteria.

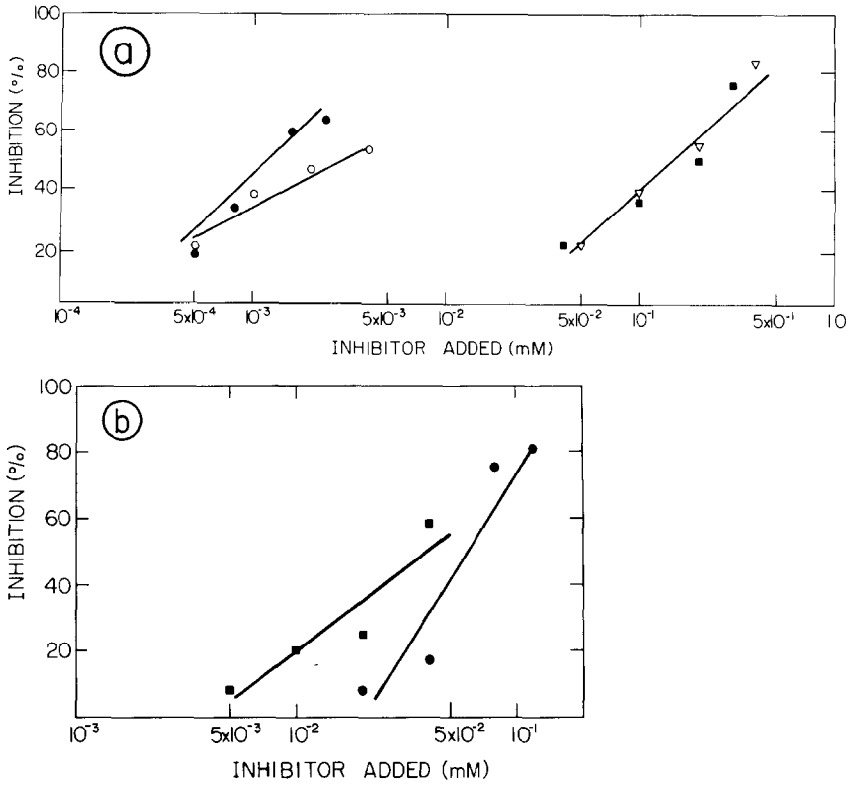

Fig. 4. Inhibition by D-mannose derivatives of aggregation of guinea pig erythrocytes by *E. coli* 346 (a) and *S. typhimurium* (b). Symbols as in Table II.

L-Mannose (**3**), 2-deoxy-D-*arabino*-hexose (**4**), α-D-mannopyranosyl phosphate (**7**), and D-mannose 6-phosphate (**2**), were all noninhibitory in the range of sugar concentrations tested (Table III). Oligosaccharides in which the D-mannosyl residues are substituted by other sugars were either noninhibitory or poor inhibitors. Thus, **24** and **25** were 4 and 1.7 times, respectively, less effective than **5**, **26** was essentially inactive at 1.0mM concentration, and **27–29** were noninhibitory at 1.6mM concentration (Table III).

K. pneumoniae. Among the sugars tested, the most potent inhibitors of yeast agglutination by *E. coli* were also the best inhibitors of yeast agglutination by *K. pneumoniae* (Fig. 3a and Table II). However, disaccharide **10**, a poor inhibitor of *E. coli*, was a moderate inhibitor of *K. pneumoniae*, whereas **15** was not as strong an inhibitor of *K. pneumoniae* as of *E. coli*.

S. typhimurium. Most of the sugars tested were either less or equally inhibitory for yeast agglutination by *S. typhimurium* as compared to **5**. The branched oligosaccharides **20** and **23** were, however, more effective inhibitors than **5**, (4 and 2 times, respectively). Among the compounds that were noninhibitory for *E. coli*, all those tested with *S. typhimurium* were also noninhibitory (Table III).

Influence of the type of target cell on the relative inhibitory potency of various sugars. — Compound **6** was the strongest inhibitor of erythrocyte aggregation by *E. coli* (125 times more than **5**), whereas the β-D anomer **8** was the weakest (Fig. 4a and Table IV). The relative inhibitory potency of **6** is the same for erythrocyte agglutination and yeast aggregation by *Salmonella*, being 2.5 times less effective than **5** (Fig. 4b and Table IV).

DISCUSSION

Measurements of the relative inhibitory activity of various carbohydrates have been used extensively to gain insight into the specificity and structure of the combining sites of lectins[20]. In the present study, the sugar specificity of *E. coli* 346, *K. pneumoniae*, and *S. typhimurium* lectins was studied by inhibition of yeast aggregation with various D-mannose derivatives. Earlier work has shown a very good correlation between yeast agglutination by bacteria and the ability of such bacteria to bind to epithelial cells[18] or macrophages[21].

Several general points concerning the specificity of the bacterial lectins examined can be made: (a) The oligosaccharides in which the D-mannosyl residues are substituted by other sugars are either noninhibitory or poor inhibitors, confirming the strict specificity of the MS bacterial lectins for D-mannose. (b) The L-isomer of mannose is noninhibitory up to a concentration of 4mM. Modification of OH-2 and -6, as in **4** and **2**, respectively, caused loss of the inhibitory activity (Table III), showing the importance of these groups for binding of the sugar by the bacterial lectins, as pointed out originally for *E. coli* and *S. typhimurium* by Old[13]. (c) The relative inhibitory activities of **6** and **8**, as well as of trisaccharide **15**, in reaction mixtures containing guinea pig erythrocytes were qualitatively similar to those obtained with the yeast cells (Table IV), suggesting that the type of target cell for monitoring the MS lectin does not influence the results. (d) Although some quantitative differences were noticeable, it appears that the specificity of the isolated fimbriae is very similar to that of the intact bacteria, which agrees with other evidence[3] that fimbriae mediate the MS adherence of the bacteria to cells. This also provides justification for the use of intact bacteria, rather than fimbriae, for studies on the specificity of the fimbrial lectins. In this sense, the bacteria can be considered as immobilized lectins. (e) There are variations in the slopes of the inhibition lines for the various sugars tested (Figs. 2–4), which may be accounted for by assuming that the bacterial fimbriae or their subunits exist as a family of lectins (or isolectins) with closely related specificities, as suggested by Kisailus and Kabat[22] for the *Bandeiraea simplicifolia* lectins.

It should also be noted that the absolute concentration of **5** required for 50% inhibition of yeast aggregation by the various organisms was not the same. Thus, whereas for *S. typhimurium* this concentration was close to that of *E. coli*, a concentration higher by about one order of magnitude was required with *K. pneumoniae* (Table II). This is probably due not only to differences in the affinities

of the lectins for **5**, but also to their mode of presentation on the bacterial surface.

Further analysis of the results revealed that there are two types of sugar specificity of the bacterial lectins, as judged from the pattern of the relative inhibitory activity of the various D-mannose derivatives. One pattern of inhibition was observed with *E. coli* and *K. pneumoniae*, and another with *S. typhimurium*. The difference is best illustrated by the observation that, whereas with the former lectins **6** and **15** are much better inhibitors than **5**, with *S. typhimurium* the inhibitory activity of the three compounds is nearly the same. With *E. coli*, several of the branched oligosaccharides, as well as the aromatic glycoside **6**, were excellent inhibitors, being 30 times more effective than **5**. Trisaccharide **15** was also a very good inhibitor, being 21 times more inhibitory than **5**. However, a change in the position of the glycosidic bond between the two D-mannose units [resulting in the trisaccharide **16**], or addition of one or two D-mannosyl residues to trisaccharide **15** (resulting in the corresponding tetrasaccharide **17** and pentasaccharide **18**, respectively), caused a decrease of 30–48 times in the inhibitory potency. Removal of a single D-mannosyl residue from trisaccharide **15** (resulting in disaccharide **14**) caused a decrease of more than 50-fold in the inhibitory potency. Disaccharides **10**–**12**, as well as trisaccharide **13**, were also weak inhibitors, in the same range as **5**.

Our results indicate that the combining sites of the *E. coli* and *K. pneumoniae* lectins are probably in the form of an extended pocket on the surface of the lectin, corresponding to the size of a trisaccharide. It is possible that the strong inhibitor, trisaccharide **15**, assumes in solution a preferred conformation close to that found for the same compound in the crystalline state[23]. In this conformation, the D-mannopyranose and the D-glucopyranose rings have the normal $^4C_1$ (D) conformation, and an intramolecular bond O-3″-H . . . O-5′ stabilizes the position of the terminal 2-acetamido-2-deoxy-D-glucopyranose residue in relation to the β-D-mannopyranosyl residue. If this is so, we may tentatively assume that the combining sites of the *E. coli* and *K. pneumoniae* lectins fit best the surface of this trisaccharide, making close contacts with OH-2, -3, -4, and -6 of the terminal, nonreducing α-D-mannopyranosyl group, and additional contacts with the terminal, reducing 2-acetamido-2-deoxy-D-glucose residue, and perhaps also with the region of the hydrogen bond just mentioned above. Since the D-mannose disaccharides tested are not better inhibitors than **5**, it is unlikely that the lectins interact strongly with the subterminal β-D-mannopyranosyl residue of the trisaccharide. The finding that **6** is a strong inhibitor for the *E. coli* and *K. pneumoniae* lectins suggests the presence of a hydrophobic region in the combining site or close to it. The occurrence of such regions in several plant lectins is well documented[24]. Interactions with the hydrophobic aglycons, however, are not sufficient by themselves for binding to these lectins, since **8** is only weakly inhibitory.

The reasons for the moderate or strong inhibitory activity of the branched oligosaccharides (**19**–**23**) is not clear, especially as they do not contain the β-D-Man*p*-(1→4)-D-GlcNAc residue. It may perhaps be due to a statistical (entropic) effect or to suitable positioning or clustering of several of the D-mannose residues.

The sugar specificity of *S. typhimurium*-surface lectin is markedly different from that of *E. coli* and *K. pneumoniae* lectins. In particular, the lectin of *S. typhimurium* appears to be devoid of an hydrophobic region of the type discussed earlier. Moreover, from the evidence obtained, it is impossible to say whether the sugar-binding site of *S. typhimurium* is large or small.

It is apparent that the specificity of the bacterial lectins described in this study is quite different from that of concanavalin A, a well characterized lectin with closely related sugar-specificity[3,4]. Firstly, whereas the latter is inhibited by both D-mannose and D-glucose, the bacterial lectins are not inhibited by D-glucose[3,4]. Secondly, **10** is a considerably better inhibitor of concanavalin A than is **5**, whereas there is little difference between the inhibitory activity of these two sugars on yeast agglutination by *E. coli*. Also, concanavalin A binds well *N*-acetyllactosamine-type oligosaccharides[25], which are poor inhibitors of the *E. coli* lectin.

A more detailed description of the combining site of the *E. coli* and *K. pneumoniae* lectins, on the one hand, and of the *S. typhimurium* lectin on the other, must await the availability of additional data on the specificity of the lectins, and especially of thermodynamic data on their interaction with various sugars. Whatever the exact shapes of the combining site of the bacterial MS lectins are, it is clear that they bind very well to structures of the type found in oligomannoside units of glycoproteins, including those that are present on cell surfaces[26], strongly suggesting that such structures serve as the receptors on epithelial cells for the adherence of MS bacteria.

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