[33] *Escherichia coli* Surface Lectins

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The ability of D-mannose and some of its derivatives to inhibit the attachment of *Escherichia coli* and other enteric bacteria to eukaryotic cells was first demonstrated in the 1950s,[1] and was found to be widespread among different pathogenic bacterial strains tested[2–4] (e.g., 46% of *E. coli,* 44% of *Klebsiella pneumonia,* and 100% of *Citrobacter diversus*). It was suggested[5] that lectin-like proteins (adhesins) on the outer surface of the bacteria are responsible for their binding to cells via mannosyl residues located on the surface of the latter. Mannose-specific lectins of *E. coli* have been isolated in the form of either bacterial flagella[6,7] or fimbriae (pili)[8–10] and found to be composed of protein subunits of molecular weight approximately 36,500 and 17,000, respectively. Mannose-binding activity was also found to be associated with the outer membranes of *E. coli,* but the corresponding lectin has not yet been identified.[11]

Assay Method

Principle. The mannose-specific activity of the *E. coli* lectins is determined by their ability to agglutinate *Saccharomyces cerevisiae* cells,[4] and the specificity of this reaction is further ascertained by its inhibition in the presence of methyl α-D-mannoside. Two types of assay are used: semiquantitative determination of agglutination on microscope glass slides and quantitative determination of the agglutinating activity in microtiter plates.

Preparation of Yeast Cells. Saccharomyces cerevisiae is grown with shaking for 16 hr at 37° in a medium (1 liter) containing peptone (2%, Difco), yeast extract (1%), and glucose (2%). The cells are harvested by

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[5] I. Ofek, D. Mirelman, and N. Sharon, *Nature (London)* **265,** 623 (1977).
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[7] Y. Eshdat, N. Sharon, I. Ofek, and D. Mirelman, *Isr. J. Med. Sci.* **16,** 479 (1980).
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[10] Y. Eshdat, F. J. Silverblatt, and N. Sharon, *J. Bacteriol.* **148,** 308 (1981).
[11] Y. Eshdat, V. Speth, and K. Jann, *Isr. J. Med. Sci.* **16,** 479 (1980).

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centrifugation at 1500 *g* for 10 min, suspended in 1 liter of phosphate-buffered saline (5 m*M* potassium-sodium phosphate buffer, pH 7.4, in 150 m*M* NaCl, PBS), and sedimented again. After removal of the supernatant the pellet is weighed, suspended in PBS [1 g (wet weight)/10 ml] in 150-ml Corex bottles and incubated for 1 hr at 23° with glutaraldehyde (Merck; 1 mg/ml). The glutaraldehyde-fixed cells are washed twice with 125 ml of PBS by centrifugation, incubated for 30 min at 23° with glycine (10 mg/ml) in PBS, and washed twice as above. The cells are stored at 4° as a suspension in PBS [25 mg (wet weight)/ml] containing 0.02% NaN_3 and are stable for at least 6 months.

Agglutination Assay. For semiquantitative assay, 5–20 μl of protein (1–5 mg/ml) or bacterial suspension (10^8 cells/ml), in PBS or in 5 m*M* Tris-HCl buffer, pH 8.0, are mixed on a microscope glass slide with 20 μl of the yeast suspension. Agglutination is observed within 2–5 min. The specificity of agglutination is tested by the addition of 5 μl of 0.5 *M* methyl α-D-mannoside (Pfanstiehl) to the bacteria before the addition of the yeasts.

For quantitative assay, twofold dilutions of the lectin solution or bacterial suspension (25 μl) are prepared in microtiter plates with flat-bottom wells, in the presence or the absence of 0.25 *M* methyl α-D-mannoside. The yeast suspension (25 μl) is added to each well, the plates are gently shaken on a rotary shaker (150 rpm) for 10 min, and the cells are allowed to settle for 1–2 hr at room temperature. The agglutination is monitored microscopically using an inverted microscope (Unicon Mic B1, × 100), and the minimal protein concentration that is still capable of agglutinating the yeasts in the assay conditions is thus determined.

Protein Determination. Protein concentration is determined by the method of Markwell *et al.*,[12] which is a modification of the method of Lowry *et al.*[13] for proteins that are difficult to dissolve. Bovine serum albumin (Pentex) is used as a standard.

Purification of *E. coli* Strain 7343 Mannose-Specific Lectin

Growth of Bacteria. Escherichia coli strain 7343 (isolated from a urinary tract infection at Sheba Hospital, Ramat Gan, Israel) is used.[6] The bacteria are grown in stationary cultures for 48 hr at 37° in a medium (2 liters) containing bactopeptone (1.0%), yeast extract (0.5%), and NaCl (0.5%); the same medium, but with the addition of 1.5% agar, is used for storage of the bacteria on slants at 4° with monthly transfers. The cells grown in

[12] M. A. K. Markwell, S. M. Hass, L. L. Bieber, and N. E. Tolbert, *Anal. Biochem.* **87**, 206 (1978).

[13] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).

the liquid medium are harvested by sedimentation at 12,000 *g* for 20 min at 4°, suspended in 500 ml of cold 0.5% NaCl, and recentrifuged as above. The yield is approximately 2.6 g, wet weight, of cells.

Isolation of Lectin. The washed *E. coli* 7343 cells are suspended in 30 ml of 5 m*M* phosphate buffer, pH 7.4, made 1 *M* in NaCl and containing 0.03 m*M* phenylmethanesulfonyl fluoride (Sigma) to prevent protease activity, and agitated in a Sorvall Omnimixer (setting 5, 30 min, 0°). The bacteria are collected by centrifugation at 10,000 *g* for 20 min, agitated again under the same conditions, and reprecipitated as above. The homogenized bacteria should no longer be capable of agglutinating yeast cells at concentrations of up to 10^{10} bacteria per milliliter.

The combined supernatant is centrifuged twice at 6000 *g* for 10 min to remove any remaining bacteria. High speed centrifugation (50,000 *g*, 3 hr) affords a pellet (5 mg of protein) that gives complete agglutination of yeast cells down to a concentration of 300 μg/ml.

The lectin in the 50,000 *g* pellet can be further purified by gel filtration on a column of Sepharose CL-4B (1.5 × 85 cm) and elution with 0.1 *M* Tris-HCl buffer (pH 8.0) containing 0.1 *M* NaCl and 1 m*M* EDTA. Most of the protein is eluted as a single homogeneous peak at the void volume of the column. The fractions comprising this peak are combined, dialyzed against water, and lyophilized. The protein is obtained in about 90% yield and possesses the same yeast agglutinating activity as that applied to the column.

Purification of *E. coli* Strain 346 Mannose-Specific Lectin

Growth of Bacteria. Escherichia coli strain 346,[14] a clinical isolate that exhibits mannose-specific attachment to buccal epithelial cells and to yeast cells, was obtained from Dr. F. J. Silverblatt (VA Medical Center, Sepulveda, California); it is stored at 4° on slants containing Trypticase soy broth (Biolife) and 1.5% agar, and transferred once a month. For preparation of the lectin, the bacteria are grown in stationary cultures in Trypticase soy broth (4 liters) for 48 hr at 37°. The bacterial cells are harvested by centrifugation at 10,000 *g* for 10 min at 4°. They are suspended gently in 700 ml of 0.5% NaCl and recentrifuged at 7500 *g* for 10 min to yield 10 g (wet weight) of cells.[15]

Isolation of Lectin. The washed bacteria are suspended in 5 m*M* Tris-HCl buffer (pH 8.0) containing 30 μ*M* phenylmethanesulfonyl fluoride,

[14] F. J. Silverblatt and L. S. Cohen, *J. Clin. Invest.* **64**, 333 (1979).

[15] Other strains of *E. coli* were used by other laboratories for purification of fimbriae [Salit and Gotschlich[8]; J. C. McMichael and J. T. Ou, *J. Bacteriol.* **138**, 969 (1979); T. K. Korhonen, E. L. Nurmiaho, H. Ranta, and C. Svanborg-Eden, *Infect. Immun.* **27**, 569 (1980)] with properties very similar to the fimbrial bacterial lectin described in this procedure.

and agitated for 20 min at 0° in a Sorvall Omnimixer (setting 4). Centrifugation at 7000 *g* for 10 min gives a pellet and a supernatant; the specific yeast agglutinating activity of the bacteria in the pellet is approximately one-third of that of the starting bacteria. The supernatant is centrifuged twice at 6000 *g* for 10 min to remove residual bacteria. The lectin is purified from the supernatant by one of two methods.

METHOD 1. The supernatant is centrifuged twice at 31,000 *g* for 10 min to remove residual membrane vesicles and flagella and then centrifuged at 200,000 *g* for 3 hr. The pellet, suspended in the Tris buffer, is capable of agglutinating yeast cells down to a concentration of 50 μg/ml.

METHOD 2. A solution of 1 *M* $MgCl_2$ is added dropwise with continuous mixing to the supernatant, to give a final concentration of 0.1 *M* $MgCl_2$. After 4 hr at 0°, the turbid solution is centrifuged at 12,000 *g* for 10 min, and the pellet is suspended in 35 ml of the Tris buffer. After centrifugation at 31,000 *g* for 10 min to remove residual membranes and flagella, 4 ml of 1 *M* $MgCl_2$ are added to the supernatant with continuous mixing; the solution is kept for 4 hr at 0° and then recentrifuged at 12,000 *g* for 10 min. The

AMINO ACID COMPOSITION OF *Escherichia coli* MANNOSE-SPECIFIC LECTINS[a]

Amino acid	Strain 7343 flagellar lectin (mol %)	Strain 346 fimbrial lectin (mol %)
Aspartic acid	14.7	13.5
Threonine	8.5	12.1
Serine	9.5	6.2
Glutamic acid	10.1	8.7
Proline	2.2	1.6
Glycine	6.9	9.8
Alanine	13.2	16.8
Cysteine	<0.1	1.1
Valine	6.5	9.1
Methionine	1.2	<0.1
Isoleucine	5.2	3.2
Leucine	8.2	6.4
Tyrosine	1.9	2.2
Phenylalanine	2.2	3.6
Tryptophan	<0.1	<0.1
Lysine	5.1	3.0
Histidine	0.5	1.1
Arginine	4.1	1.6

[a] The amino acid composition was determined according to Eshdat *et al.*[6]

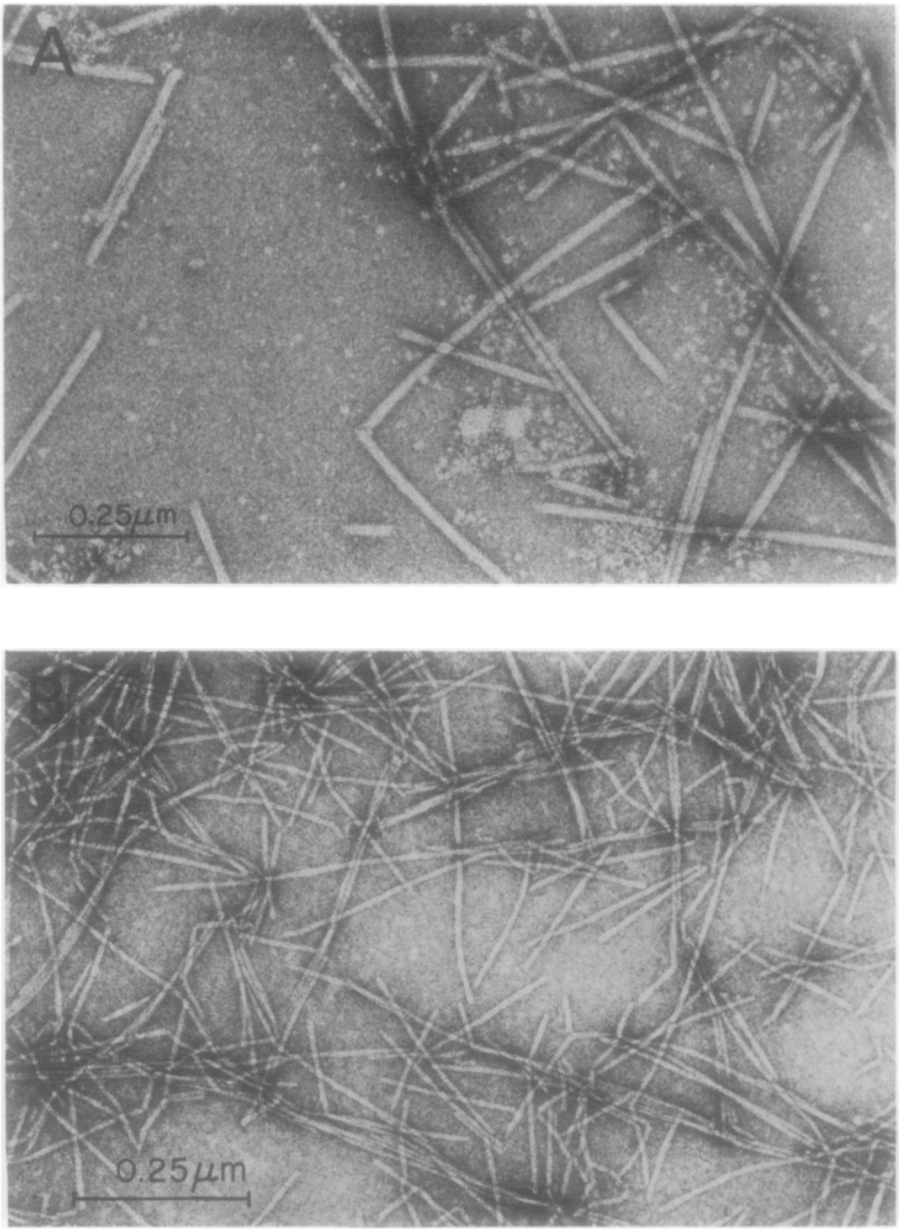

FIG. 1. Electron micrographs of (A) mannose-specific flagella isolated from *Escherichia coli* 7343; (B) mannose-specific fimbriae isolated from *Escherichia coli* 346. Samples were negatively stained with 0.5% uranyl acetate on copper grids coated with Parlodion and carbon and examined in a Philips EM 300 electron microscope.

above precipitation procedure is repeated three times. The last pellet obtained by centrifugation at 12,000 *g* is suspended in the Tris buffer to give a concentration of 2 mg of protein per milliliter. The yeast agglutinating activity of the suspended pellet is essentially the same as that of the lectin obtained by the preceding procedure.

Properties

Two mannose-specific lectins were isolated from two different pathogenic strains of *E. coli* obtained from patients having a urinary tract infection. Both lectin-like proteins are located at the outer surface of the bacteria and mediate their adherence to eukaryotic cells. This adherence, as measured by the yeast agglutination assay, is inhibited by D-mannose and some of its derivatives, such as methyl and *p*-nitrophenyl α-mannosides (down to concentrations of approximately 0.6 m*M* and 10 μ*M*, respectively), but not by other sugars, such as D-glucose and D-galactose.[6] However, the two lectins differ in their amino acid composition (see the table), molecular structure, ultrastructure (Fig. 1), and physicochemical properties.

The lectin from *E. coli* 7343 is isolated as flagella comprised of identical protein subunits of M_r 36,500.[6] This value is lower than that reported for most *E. coli* flagella. Moreover, unlike typical bacterial flagella, the mannose-specific flagella exhibit structural stability when heated at 80° for 1 hr, while retaining their yeast agglutinating activity, and are only partially dissociated at pH 3.[7] The purity of the lectin can be determined by gel electrophoresis in the presence of SDS[6] and by its ultrastructure as observed by electron microscopy (Fig. 1A).

The lectin isolated from *E. coli* 346 is organized as hairlike appendages, named fimbriae or type 1 pili.[1,9] It is composed of protein subunits (M_r 16,600)[10] arranged in a highly stable array. Unlike the flagellar lectin, the mannose-specific fimbriae cannot be dissociated into subunits by treatment with urea or sodium dodecyl sulfate.[16] Dissociation of the *E. coli* 346 fimbriae into monomeric subunits can be achieved by incubation with saturated guanidine hydrochloride. Upon removal of the latter by dialysis, active subunits are obtained that exhibit mannose-binding activity and can in part be reassembled into fimbrial structures.[10] The purity of the lectin can therefore be determined by gel electrophoresis in SDS after its dissociation by guanidine hydrochloride[10] and by electron microscopy (Fig. 1B).

[16] Treatment of the mannose-specific fimbriae preparations with 10 *M* urea is helpful in removing residual flagella that are not removed by centrifugation at 31,000 *g* or in isolating fimbriae from heavily flagellated bacteria.