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Association of Alginate from *Pseudomonas aeruginosa* with Two Forms of Heparin-Binding Lectin Isolated from Rat Lung

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An endogenous heparin-binding lectin activity isolated from rat lung was separated into two distinct isolectin forms which showed subtle changes in carbohydrate specificity. The two lectin forms displayed different specificities toward alginic acid-purified cystic fibrosis isolates of *Pseudomonas aeruginosa* when assayed by inhibition of both hemagglutination and [^{3}H]heparin binding. This ability of isolectin forms to show higher affinity toward alginic acid from certain *P. aeruginosa* strains may suggest that there is a selective mechanism in the colonization of patients with cystic fibrosis.

Fibronectin, laminin,and endogenous lectins are ligands which appear to function, at least in part, by allowing specific adhesions between cells of vertebrates and complex carbohydrate-containing macromolecules such as glycosaminoglycans and mucins (1, 1a, 21). The specific association between fibronectin (4), laminin (19), and endogenous lectins (13) and bacterial species has been demonstrated, suggesting that a role may be played by host endogenous ligands in the adherence of bacteria to mucosal surfaces.

Recently, a report that extracts of adult chicken liver identical to those of heparin-binding lectin can yield a dermatan sulfate-specific lectin (9) may indicate that the heparin-binding lectin composed of two nonidentical subunits of 16,000 and 13,000 molecular weight may exist as a family of isolectins with unique specificity. The lectin from *Phaseolus vulgaris* has been divided into a family of five isolectins (L_4, L_3E_1, L_2E_2, L_1E_3, and E_4), each with specific binding properties (6, 12, 22, 23). Similar results have also been reported with *Griffonia simplicolia* lectin, which is also composed of nonidentical subunits (14, 15). Here, we present evidence that heparin-binding lectin isolated from rat lung can be separated into at least two unique forms. The two lectin forms displayed unique specificity toward alginates derived from cystic fibrosis isolates of *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Lectin extraction. Lungs from just-weaned Sprague Dawley rats were homogenized in 9 volumes (wt/vol) of MEPBS (75 mM NaCl, 75 mM Na_2HPO_4-KH_2PO_4 (pH 7.2), 4 mM β-mercaptoethanol, 2 mM EDTA) plus 1 M NaCl in a Waring blender (2, 18). Homogenates were centrifuged at 100,000 × *g* for 1.5 h, and the supernatant was collected through cheesecloth. Extracts were then chromatographed on a Sepharose Cl-2B (Pharmacia Fine Chemicals, Piscataway, N.J.) column equilibrated and eluted with MEPBS. The void and included lectin peaks were purified to homogeneity by affinity chromatograph on heparin-Sepharose as described previously (2, 18). The included lectin peak was also purified with alginate-Sepharose as an affinity ligand. Alginate prepared from *P. aeruginosa* 3313 was bound to epichlorohydrin-activated Sepharose 4B (Pharmacia) and was used in the same fashion as described previously for heparin-Sepharose (2).

Lectin inhibition assays. Lectin activity was determined by hemagglutination in microtiter V plates (Dynatech, Industries, Inc., McLean, Va.; Fisher Scientific Co., Pittsburgh, Pa.) with serial twofold dilutions of lectin extracts (25 μl) in MEPBS (25 μl) as described previously (2). In all cases, lectin purified by chromatography on Sepharose Cl-2B was used, as results of previous studies have demonstrated that the binding affinities of these preparations are identical to those of heparin-Sepharose-purified preparations (2, 18). To the dilutions was added 25 μl of saline (0.15 M NaCl) and 25 μl of 1% bovine serum albumin in saline. Hemagglutination was measured with a 4% suspension of alcohol-treated, trypsinized, glutaraldehyde-fixed rabbit erythrocytes (AGTR) (11) or with human, ovine, and bovine erythrocytes prepared as described below. Lectin inhibition was assayed by replacing saline with the hapten diluted to the desired concentration in saline. The lowest concentration of hapten that inhibited lectin activity by one step was deemed the concentration that inhibited lectin activity by 50%.

Heparin-binding assay. Heparin-binding assays were performed with Immulon 2 Removawell strips (Dynatech) following previously published techniques (3). Each well was incubated with 100 μl of Sepharose Cl-2B-purified lectin solution as described above for 16 h at 4°C. The wells were washed four times in MEPBS–1% bovine serum albumin before incubation with 0.06 μg of [^{3}H]heparin containing 19,400 cpm in 100 μl of MEPBS for 30 min at room temperature. The wells were washed four times in MEPBS, and each well was broken off and counted in a liquid scintillation center. [^{3}H]heparin (specific acitivity, 0.14 mCi/mg) was purchased from New England Nuclear Corp., Boston, Mass.

***P. aeruginosa* strains: maintenance and culture.** *P. aeruginosa* strains isolated from patients with cystic fibrosis were Homma-serotyped and kindly supplied by H. Rabin, Depart-

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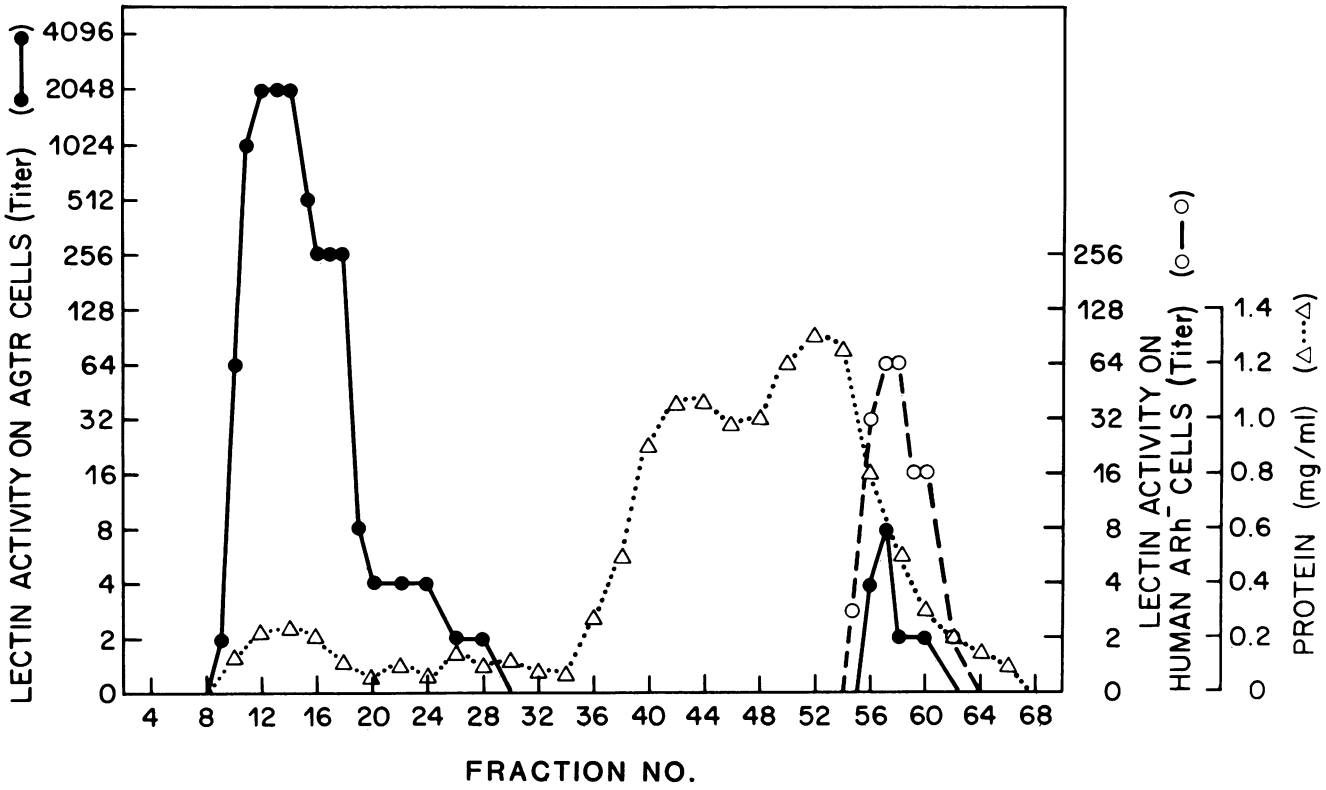

FIG. 1. Separation of rat lung isolectin forms. Crude extracts were chromatographed on Sepharose Cl-2B as outlined in the text. Two discrete peaks of lectin activity were obtained. Symbols: ●, lectin activity on AGTR cells; ○, lectin activity on human A Rh^- cells; △, protein (in milligrams per milliliter).

ment of Microbiology and Infectious Diseases, University of Calgary. All strains were cultured on pseudmonas isolation agar (Difco Laboratories, Detroit, Mich.) at 28°C for 48 h to confirm the expression of a mucoid phenotype. Thereafter, the strains were maintained on pseudomonas isolation agar slants at −70°C.

Isolation of exopolysaccharides. Exopolysaccharide was prepared as described previously (13) from cells grown in suspension in the medium described by Piggott et al. (16), except that it lacked $FeSO_4$.

Isolation of lipopolysaccharides. Lipopolysaccharide (LPS) was prepared as described previously (13) following the procedure of Darveau and Hancock (5).

Polysaccharide analyses. 2-Keto-deoxyoctulosonic acid, uronic acids, and hexosamines were assayed by standard colorimetric assays (7, 8, 10). The ratio of mannuronic acid to guluronic acid was determined by the method of Vadas et al. (20), and the degree of acetylation was determined by the method of McComb and McCready (14).

RESULTS

Separation of lectin forms. Heparin-binding lectin isolated from rat lung could easily be separated into two clearly definable fractions by passing MEPBS–1 M NaCl extracts through a Sepharose Cl-2B column (Fig. 1). The major fraction (form I) behaved as a large aggregate and was collected at or near the void volume of the column. A minor fraction (form II) did not aggregate and was included in the trailing edge of the major protein peak eluted off the column. The activity of both peaks agglutinated AGTR erthrocytes and was inhibited by heparin. The lectin from both peaks appeared identical to that reported previously (18) for

TABLE 1. Comparison of heparin-binding lectin forms I and II isolated from rat lung

Form	Structure	Chromatography on Sephaded G-100	Erythrocyte specificity	Inhibition by heparin
I	16,000- and 13,000-dalton peptides	Large aggregates that void	AGTR	50% of lectin titer by 0.01 mg of hapten per ml
II	16,000- and 13,000-dalton peptides	Included	AGTR and human type A	50% of lectin titer by 0.01 mg of hapten per ml

heparin-binding lectin isolated from rat lung on sodium dodecyl sulfate-polyacrylamide gels following purification on heparin-Sepharose (Table 1).

Erythrocyte specificity. The two lectin fractions were assayed for erythrocyte specificity in hemagglutination assays with trypsinized, glutaraldehyde-fixed rabbit erythrocytes; AGTR; human type A, B, and O erythrocytes that were trypsinized and formalin fixed; and ovine and bovine erythrocytes treated in the same way. The major lectin peak (form I) agglutinated only AGTR erythrocytes while the minor peak (form II) was positive in agglutination assays with either AGTR erythrocytes or human type A erythrocytes (Fig. 1, Table 1). No agglutination was seen with the other erythrocytes tested (data not shown).

Alginate specificity. It has previously been shown that alginic acid purified from certain Homma serotypes of *P. aeruginosa* failed to inhibit the agglutination of AGTR erythrocytes by form I of the lectin but that alginate prepared from Homma nontypable cystic fibrosis isolates acted as a competitive inhibitor of lectin activity (13). The ability of alginates from the same strains of *P. aeruginosa* to inhibit form II of the lectin was tested in hemagglutination assays with both AGTR and type A human erythrocytes (Table 2). Form II differed from form I with respect to its specificity for alginate in agglutination studies. Alginate from both Homma-typable strains (492A and 3313) and -nontypable strains (842 and 890) were potent inhibitors of form II activity. Only alginate derived from Homma-nontypable strains proved to be good inhibitors of agglutination by form I of the lectin (13). The mannuronic acid-containing polymer isolated from *Arthrobacter viscosus* (13) failed to inhibit either form I or form II of the lectin (Table 2). As with the inhibition studies with form I (13), LPS proved to be a very weak inhibitor of form II of the lectin (Table 2).

Heparin-binding assay. Alginate specificity of forms I and II of the lectin was also tested in a competitive binding assay with [^{3}H]heparin. The results of these experiments corroborate results of the hemagglutination inhibition assays (Fig. 2). Alginates prepared from *P. aeruginosa* 842 and 890 were more potent inhibitors of form I of the lectin when compared with alginate prepared with the Homma-typable strains 492A and 3313. However, form II of the lectin did not differentiate between alginates prepared from these strains.

TABLE 2. Hapten inhibition of heparin-binding lectin form II isolated from rat lung

Addition	Homma serotype	Mannuronic: guluronic ratio[a]	Hapten concn (mg/ml) producing 50% inhibition in the following erythrocytes:	
			AGTR	Human type A
A. viscosus			>1	>1
P. aeruginosa 492A alginate	M	5.25	3×10^{-4}	6×10^{-4}
P. aeruginosa 492A LPS			>1	>1
P. aeruginosa 3313 alginate	B	3.35	6×10^{-4}	3×10^{-4}
P. aeruginosa 3313 LPS			>1	>1
P. aeruginosa 842 alginate	NT[b]	3.00	6×10^{-4}	6×10^{-4}
P. aeruginosa 842 LPS			>1	>1
P. aeruginosa 890 alginate	NT	4.00	1.5×10^{-4}	3.0×10^{-4}
P. aeruginosa 890 LPS			>1	>1

[a] Moles of mannuronic acid per moles of guluronic acid.
[b] NT, Nontypable.

DISCUSSION

Adhesion to mucosal surfaces is recognized as an important component of the pathogenic mechanism leading to the colonization of host tissue. Although the majority of attention has been paid to bacterial ligands involved in this process, recent studies have identified endogenous host ligands that are capable of specific bacterial interactions (4, 13, 19). We have previously reported that endogenous heparin-binding lectin isolated from rat lung interacted with alginic acid purified from Homma nontypable cystic fibrosis isolates of *P. aeruginosa*, but not with representatives of Homma serotypes G, M, B, and I (13). Here, we present evidence that, like many other lectins, the heparin-binding lectin isolated from rat lung can be separated into a family of isolectin activities. The two major forms of the lectin compared in this study showed unique specificity for alginate prepared from both Homma-typable and -nontypable strains of *P. aeruginosa*. These properties held true in both hemagglutination inhibition studies and in heparin-binding studies. This represents an important finding, as it demonstrates that the alginate derived from *P. aeruginosa* can, in fact, compete with host secretions for lectin-binding sites and marks a significant advance over the hemagglutination assay for assessing lectin-alginate interactions. The degree of specificity determined by hemagglutination inhibition and [^{3}H] heparin-binding inhibition was different, although it was distinct in both cases. The reduction in magnitude of inhibition likely resides in the difference in the nature of the ligands being bound on the AGTR cells and heparin. This preference may represent the recognition of specific epitopes present in various concentration on alginates prepared from different strains. We recently have reported the isolation of a monoclonal antibody that shows the same alginate-binding preference as the major lectin form (8a). Similarly, Pier et al. have reported the immunological distinction of specific epitopes in alginate. Recent studies carried out by Marcus and Baker have demonstrated that lectin supplied by us from rat lung and human tissue facilitated *P. aeruginosa* adherence in their adhesion assay (H. Marcus and N. R. Baker, personal communication). Results of these studies again suggest that there is a possible role for endogenous lectins in the process of bacterial adherence and colonization. Ramphal and Pier (17) have also demonstrated that there are host receptors for alginate which stimulate *P. aeruginosa* adhesion and may allow for the bacterial association of added alginate. Endogenous lectins, as described in this report, could function as such an adhesion mechanism and could allow for the association of host extracellular polymers with bacterial polymers for the adhesion of bacteria to host tissues, allowing bacterial escape from the host immune system.

It has been suggested that patients with cystic fibrosis harbor specific serotypes of *P. aeruginosa* through the course of infection, in spite of being exposed to many other strains. It is interesting to speculate that a selective advantage to certain strains of *P. aeruginosa* may exist if patients were to possess different isolectin profiles in their lungs. This

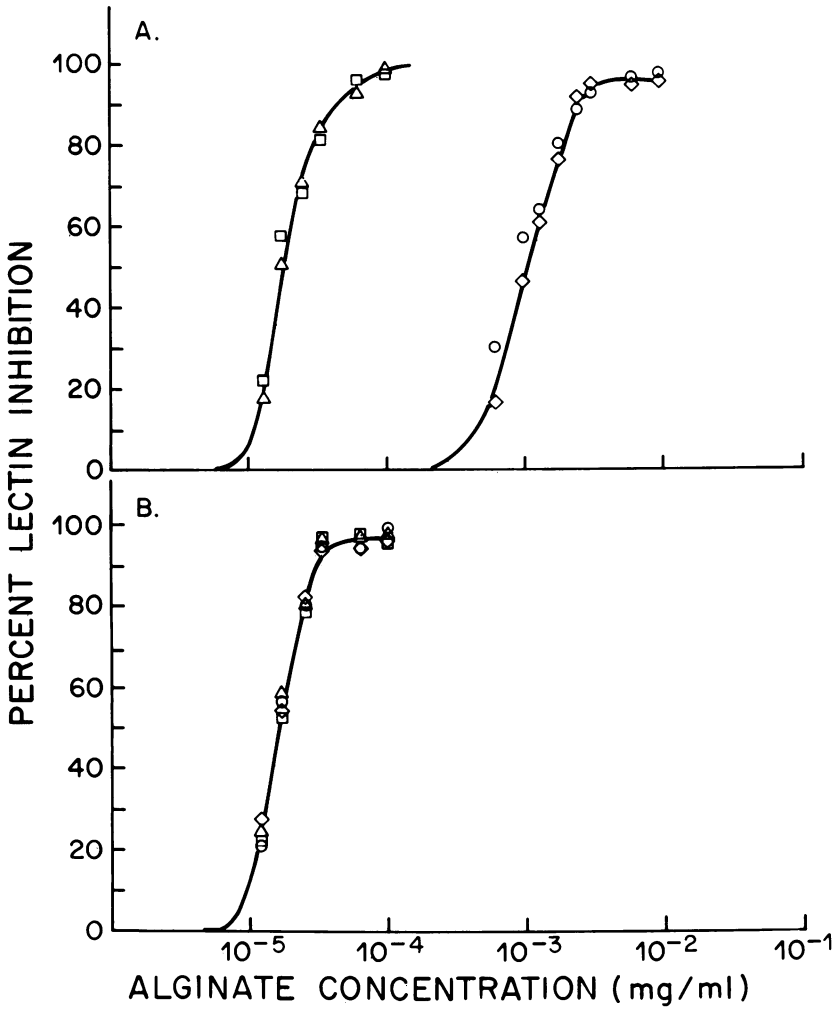

FIG. 2. Inhibition of [^{3}H]heparin binding by alginic acid. The specificity of form I (A) and form II (B) for alginic acid was determined by competitive binding assays with [^{3}H]heparin. Conditions for the assay are given in the text. Data points represent the mean of three replicate samples from a single experiment. Similar results were obtained in a minimum of three separate experiments. Symbols: □, strain 842; △, strain 890; ○, strain 3313, ◇, strain 492A.

could then facilitate the colonization of the lungs by *P. aeruginosa* that possess alginate with specific epitope distributions.

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