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MAMMALIAN KIDNEY LECTIN*

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ABSTRACT

Lectins that agglutinate intact red blood cells of rabbit, and their endogenous inhibitors, were found in extracts of cow, pig, rat, and human kidneys. Lectins were separated from inhibitors by gel chromatography on a column of Toyopearl HW-75. Adsorption tests with immobilized glycoconjugates and ion-exchange gels revealed that the bovine kidney lectin binds with sialylglycoproteins by sugar-specific interactions but not by nonspecific, electrostatic interactions. The results of the hemagglutination-inhibition tests showed that all of the endogenous inhibitor fractions were active toward all renal lectins tested, and that glycoproteins having sialyl residues, and also heparin, are also good inhibitors of the lectins. However, the hemagglutination-inhibitory activity of human urinary Tamm–Horsfall glycoproteins varied from source to source, and that having the highest inhibitory activity did not always have the highest content of sialic acids. Crude bovine kidney lectin was further purified by ion-exchange chromatography on DEAE-Sephadex A-50. Analysis by SDS–polyacrylamide gel electrophoresis showed that the purified lectin consists of a ~63,000 daltons subunit.

INTRODUCTION

Various lectins are known to exist in mammalian tissues and organs, the most extensively studied of which are D-galactose/2-acetamido-2-deoxy-D-galactose-specific lectin from liver[1–3], mannan-binding protein from liver[4,5] and plasma[6], mannose 6-phosphate-specific lectin from liver[7], and β-D-galactoside-specific lectins from various tissues[8–10]. The hepatic lectin specific for D-galactose/2-acetamido-2-deoxy-D-galactose is implicated in the clearance of asialoglycoproteins from plasma, while others may participate in cell–cell recognition. In kidneys, a mannose 6-phosphate-binding protein[11] and laminin[12] (a heparin-inhibitable lectin[13]) have been detected in kidneys.

In this study we found that lectins and endogenous inhibitors exist in the

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extracts of cow, pig, rat, and human kidneys and cross-react with each other. The specificities of these lectins were examined for a number of sugars, glycoproteins, and glycosaminoglycans. These renal lectins differ from mannose 6-phosphate-binding protein and laminin in their ability to agglutinate intact red blood cells of rabbit and their high affinity for sialyl residues of glycoproteins. They also appear to be specific for sugar residues besides sialic acid, however, because samples of Tamm–Horsfall urinary glycoproteins having the highest inhibitory activity toward the lectins did not always have the highest content of sialic acid.

EXPERIMENTAL

Materials. — Fresh kidneys of cow and pig were obtained from local slaughterhouses. Rat kidneys were obtained from 8-week-old male Fisher rats. Sections of human kidney were obtained 4 h post mortem from a female patient who died of lymphoma. Toyopearl HW-75 and DEAE-Toyopearl 650 were purchased from Toyo Soda Manufacturing Co. (Tokyo, Japan), Sepharose 4B, DEAE-, SP-, and CM-Sephadexes were from Pharmacia Fine Chemicals, and CM-Cellulofine was from Chisso Co. (Tokyo, Japan). Calf fetuin and ovomucoid were purchased from Sigma Chemical Co. Bovine submaxillary mucin was purified by the method of Tettamanti and Pigman[14], porcine submaxillary mucin by the method of Katzman and Eylar[15], and porcine thyroglobulin by the method of Ui and Tarutani[16]. Tamm–Horsfall glycoproteins were purified from adult urine by the method of Goodal and Marshall[17]. Colominic acid and alginic acid were obtained from Seikagaku Kogyo Ltd. (Tokyo, Japan). Dermatan sulfate and hyaluronic acid from rat skin were prepared as described previously[18]. Chondroitin sulfate A from whale cartilage[19] and chitin sulfate from the tests of tunicates[20] were prepared as described previously.

Immobilization of glycoconjugates. — All of the ligands were immobilized on an epoxy-activated carrier or its derivatives. Bovine kidney inhibitor-fraction and calf fetuin were immobilized on formyl–spacer–Sepharose 4B by reductive amination[21]. Bovine submaxillary mucin was immobilized on hydrazino-Sepharose 4B (ref. 22) with the aid of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. Thyroglobulin was directly coupled to epoxy-activated Sepharose 4B (ref. 23). Heparin was immobilized on hydrazino-Toyopearl HW-65 with the aid of $NaCNBH_3$ (ref. 24).

Adsorption assay of bovine kidney lectin. — Immobilized-glycoconjugate gels were packed into Sepacol mini columns (Seikagaku Kogyo) to a height of 1 cm, and the columns were each washed with 10 column-volumes of 10mM Tris–HCl (pH 7.2) containing 0.1M NaCl and 4mM 2-mercaptoethanol (MTB). The kidney extract (10 mL) was applied to the columns at the flow rate of 2 mL/h at room temperature, and the columns were then washed again. One column-volume of 4% human erythrocyte suspension was applied to each column, the columns were washed, and the adsorption of the lectin was judged in terms of the red color of the adsorbed

erythrocytes (++, red; +, pink; −, white). In the case of ion-exchange gels, 20 mg of gels were suspended in 100 μL of the lectin solution with titer 16 and incubated for 1 day at 4°. The adsorption of the lectin was then expressed in terms of the hemagglutinating activities in the supernatant solution (++, titer 0; +, titer 2–8; −, titer 16).

Lectin preparation. — Kidneys were homogenized with 4 parts (volume/weight) of acetone in a Waring blender. The resultant residues were collected by filtration on a Büchner funnel lined with filter paper. The residues were re-extracted with acetone as before, and then washed with a small amount of ethyl ether. The powder was allowed to air-dry and was stored at −20° until use. Extractions were performed by using 3 parts (volume/weight) of 75mM Na_2HPO_4/KH_2PO_4 (pH 7.2) containing 4mM 2-mercaptoethanol, 2mM EDTA, and 0.075M NaCl. After centrifugation at 30,000*g* for 1 h, 3–6 mL of the supernatant solutions were applied to Toyopearl HW-75 columns (2.5 × 47.5 cm) equilibrated with MTB, and the columns were developed with the same buffer. Fractions of 2 mL were collected and assayed for lectin activity. Further purification of bovine lectin was achieved by chromatography on DEAE-Sephadex A-50 as described in the legend of Fig. 2.

Gel electrophoresis. — Gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) was performed according to the method of Laemmli[25] using 12.5% acrylamide separating-gels. Proteins were detected by the method of Aihara[26], using a silver-stain kit from Daiich Pure Chemical Co. (Tokyo, Japan).

Hemagglutination assay. — Lectin activity was assayed with intact rabbit erythrocytes. Hemagglutinating activity was determined by using serial 2-fold dilutions of an extract or the lectin fractions in microtiter V-plates (Cooke Engineering). A 4% erythrocyte suspension (25 μL) in 10mM Tris–HCl (pH 7.2) containing 0.15M NaCl and 4mM 2-mercaptoethanol (MTBS) and 25 μL of a sample solution were mixed in each well and incubated for 1 h at room temperature. The titer was taken as the reciprocal of the highest dilution of the sample causing hemagglutination.

The inhibitory activity of carbohydrate and endogeneous inhibitor was determined by incubation of the lectin solution (titer = 2) prepared from hemagglutinating-active fractions (on Toyopearl HW-75 chromatography) with 2-fold dilutions of carbohydrate solutions or aliquots of the eluted fractions in the same chromatography for 2 h at 4°, and then addition of an erythrocyte suspension. The lowest concentration that inhibited hemagglutination completely was used to define the inhibitory activity.

Chemical analysis. — Hexose was determined by the anthrone method[27] using D-galactose as a standard. Amino sugar composition was determined by using a Hitachi 835 amino acid analyzer after hydrolysis with 4M HCl for 8 h at 100°, followed by evaporation to remove HCl. Sialic acid was determined by the periodate–resorcinol method[28]. Amino acid analyses were performed with the amino acid analyzer after hydrolysis in 6M HCl at 110° in vacuum-sealed tubes for 24, 48, and 72 h.

RESULTS AND DISCUSSION

The extracts of cow, pig, rat, and human kidneys had significant hemagglutinating activities toward intact red blood cells of rabbit, although no hemagglutination was observed at high concentrations of the samples; thus bovine extracts exhibited hemagglutinating activity only after 16-fold dilutions. Addition of 2-mercaptoethanol to the extracting solution was necessary to prevent inactivation of the lectins and to achieve the maximal extraction of the lectins, although addition of 0.1M lactose or M NaCl had no effect on the extraction, in contrast to the case with chicken liver lectin[29]. When the extract of bovine kidney was chromatographed on a column of Toyopearl HW-75, the hemagglutinating activities were detected between two major protein peaks, as shown in Fig. 1A, the specific activities of which were markedly increase. The kidney extracts from other sources gave essentially the same elution patterns by chromatography on Toyopearl HW-75, as shown in Figure 1B–D. As the recoveries of activity were 120, 150, 180, and 210% for cow, pig, rat, and human kidney lectins, respectively, it was assumed that the chromatography removed endogenous inhibitors present in the extract. Therefore, the hemagglutination-inhibition activity of the fractions was also measured, and was detected in the protein peaks of low molecular weight. For the cow kidney extract, the peak of inhibitory activity coincided with the peaks of neutral sugars as detected by the anthrone method and of silalic acid as detected by the periodate–resorcinol method (elution patterns not shown). From the chromatographic elution volumes, the renal lectins were estimated to have high molecular weights. When the bovine inhibitor fraction was chromatographed on a column of Toyopearl HW-55, it was eluted in the void volume of the column, suggesting that the inhibitor has a molecular weight of >500,000. However, further purification of inhibitors has not yet been achieved because of their instability.

The specificities of the lectins obtained were examined by adsorption tests with derivatized gels and ion-exchange gels, and by hemagglutination inhibition tests. The results of the adsorption tests are shown in Table I, together with the ligand concentrations of the gels. Under physiological salt and pH conditions, the bovine lectin was strongly adsorbed on the crude bovine inhibitor–Sepharose, fetuin–Sepharose, and DEAE-Sephadex, weakly adsorbed on bovine submaxillary mucin–Sepharose and thyroglobulin–Sepharose, but not adsorbed on heparin–Toyopearl, CM-, and SP-Sephadexes. The results indicate that the lectin recognizes the sialyl residues of the glycoproteins and the binding is due to sugar-specific interactions, not to nonspecific electrostatic interactions.

The results of the hemagglutination-inhibition tests are summarized in Table II. All of the endogenous inhibitors were active toward all of the renal lectins. Glycoproteins having sialyl residues were good inhibitors of all the lectins, although sialic acid was not inhibitory at the concentrations examined. The inhibitory activity of fetuin decreased after desialylation (data not shown). Furthermore, heparin was also a good inhibitor of all the lectins, whereas all the other glycosaminoglycans

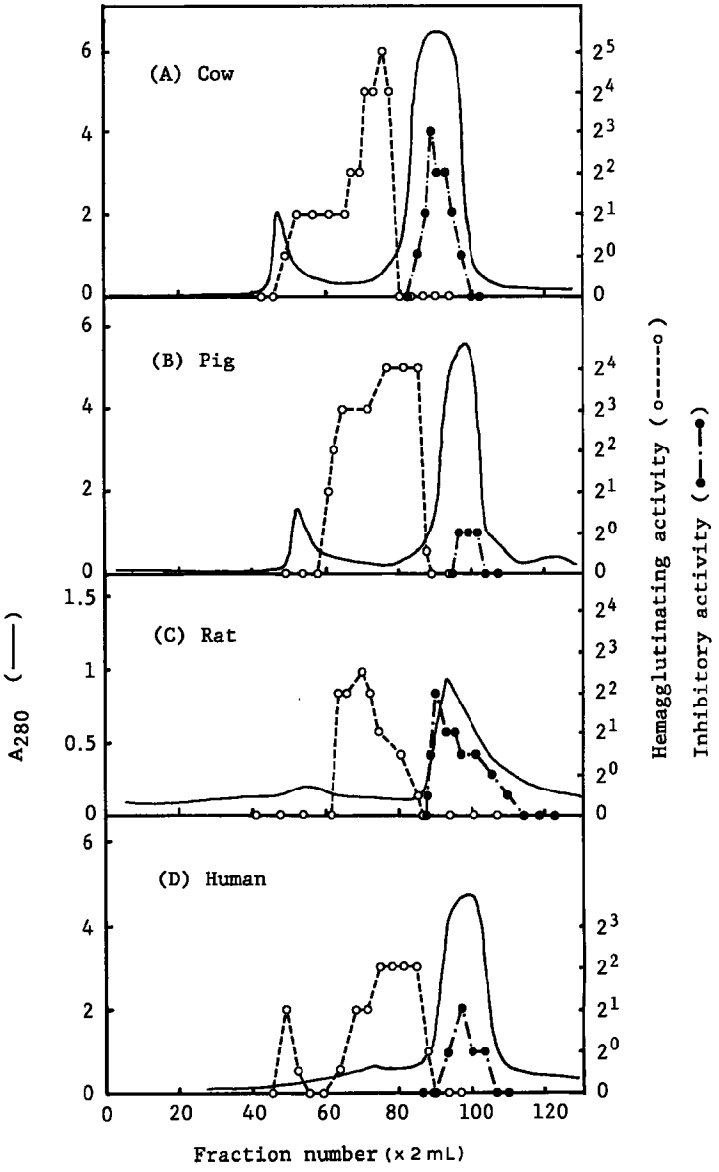

Fig. 1. Elution profiles of extracts from (A) cow, (B) pig, (C) rat, and (D) human kidneys. Gel filtration was performed on a column of Toyopearl HW-75 as described in the text. ——, protein assay; O---O, hemagglutination assay; ●-·-●, hemagglutination-inhibition assay. The hemagglutination-inhibiting activities of the eluted fractions were determined as described in the text, using the lectin solution (titer = 2) obtained by dilution of the fractions in the same chromatography that were hemagglutination-active.

TABLE I

ADSORPTION OF BOVINE KIDNEY LECTIN ONTO VARIOUS DERIVATIZED GELS

Derivatized gel	*Concentration of immobilized ligand*	*Adsorption*
Bovine inhibitor–Sepharose	12 mg/mL	++
Calf fetuin–Sepharose	87	+
Bovine submaxillary mucin–Sepharose	10	+
Thyroglobulin–Sepharose	60	+
Heparin–Toyopearl	2	−
DEAE-Sephadex	0.175 meq/mL	++
DEAE-Toyopearl	0.10	+
SP-Sephadex	0.09	−
CM-Sephadex	0.17	−
CM-Cellulofine	0.14	−

TABLE II

HEMAGGLUTINATION-INHIBITION ASSAY OF KIDNEY LECTINS

Inhibitor[a]	*Minimum conc. (mg/mL) completely inhibiting 2 hemagglutinating doses of the kidney lectins*			
	Cow	*Pig*	*Rat*	*Human*
Bovine inhibitor[b]	0.10	0.20	0.10	0.10
Porcine inhibitor[b]	0.90	1.1	0.60	0.15
Murine inhibitor[b]	0.57	0.57	0.57	—
Human inhibitor[b]	0.88	0.88	0.88	0.44
Porcine submaxillary mucin	0.23	0.057	0.11	0.11
Bovine submaxillary mucin	0.43	0.43	0.21	0.21
Heparin	0.46	1.4	0.80	0.43
Calf fetuin	0.83	0.21	0.42	0.013
Porcine thyroglobulin	3.35	3.35	1.67	3.35

[a]The following carbohydrates showed no inhibitory activity toward the lectins at the concentration indicated in parentheses: L-Fuc (43mM), Man (41mM), GalNAc (36mM), GlcNAc (30mM), Man 6-P (18mM), GlcA (35mM), NeuAc (22mM), lactose (19mM), melibiose (22mM), mannan (7.1 mg/mL), colominic acid (11 mg/mL), dermatan sulfate (18 mg/mL), chondroitin sulfate A (24 mg/mL), hyaluronic acid (16 mg/mL), alginic acid (18 mg/mL), chitin sulfate (22 mg/mL) and ovomucoid (26 mg/mL). [b]Samples were prepared from fractions active on gel chromatography (Fig. 1) by dialysis against water and subsequent lyophilization.

tested failed to inhibit the lectins. The results also indicate that the lectins bind with the carbohydrates by sugar-specific interactions but not by nonspecific electrostatic interactions. The discrepancy in the results for heparin between adsorption tests and the hemagglutination inhibition assay may be because the ligand concentration of heparin–Toyopearl is too low to adsorb the lectin, or because Toyopearl gel has a poor adsorption capacity as an affinity carrier.

The inhibititory activities and sugar compositions of urinary Tamm–Horsfall

glycoproteins are shown in Table III. Sugar compositions of Tamm–Horsfall glycoproteins differed from source to source, whereas amino acid compositions of all the glycoproteins (data not shown) were similar to that reported by Fletcher *et al.*[30]. The glycoproteins having the highest inhibitory activity did not always have the highest content of sialic acid and had a rather high ratio of 2-amino-2-deoxy-D-galactose/2-amino-2-deoxy-D-glucose. The results show that the renal lectins recognize not only sialyl residues but also other sugar residues or certain structures containing sialic acid. As Tamm–Horsfall glycoprotein is synthesized in the kidney and secreted in urine, the endogenous inhibitors were assumed to be identical to Tamm–Horsfall glycoprotein. However, this assumption was invalidated by their different solubilities in salt solutions.

The renal lectins resemble laminin in their heparin-binding ability and high molecular weights, but differ from it in other properties. The renal lectins can be extracted with aqueous solutions without detergents, but laminin cannot[28]. The renal lectins have a higher affinity to sialylglycoproteins (Table II) than laminin[13]. The renal lectins agglutinate intact rabbit erythrocytes, whereas laminin agglutinates only the trypsin- and glutaraldehyde-treated erythrocytes and not intact cells[13].

Furthermore, hemagglutinating-active fractions of bovine kidney obtained by gel chromatography (Fig. 1A) were subjected to ion-exchange chromatography on a column of DEAE-Sephadex A-50. The hemagglutinating activity was retained on the column and eluted with acetate buffer of pH 6.0, as shown in Fig. 2. An electrophoretogram of SDS–polyacrylamide gel electrophoresis is shown in Fig. 3. The purified bovine kidney lectin gave a major band corresponding to mol. wt. ~63,000, as shown in Fig. 3. The amino acid composition of the purified lectin is presented in Table IV. This lectin is rich in serine, glutamic acid, and glycine, and contains no cystine and methionine. The results indicate that bovine kidney lectin and laminin[31] differ from one another in the size of subunit and amino acid composition.

The reversible binding between the renal lectin and endogenous inhibitors under physiological conditions might be essential for their biological roles. The

TABLE III

SUGAR COMPOSITIONS AND HEMAGGLUTINATION-INHIBITION ACTIVITIES OF TAMM–HORSFALL GLYCOPROTEINS

Tamm–Horsfall glycoprotein	*Sugar composition (w/w %)*				*Minimum amount inhibiting 2 hemagglutinating doses*
	GlcN	*GalN*	*Sialic acid*	*Neutral sugar*	
I.M.	10.1	0.71	5.6	13.5	0.35
T.K.	12.8	0.80	5.0	13.9	0.36
N.S.	12.8	0.43	5.8	14.2	>4.4
T.N.	5.7	0.37	5.0	12.9	>4.2
N.I.	16.5	0.62	5.0	15.2	>4.5

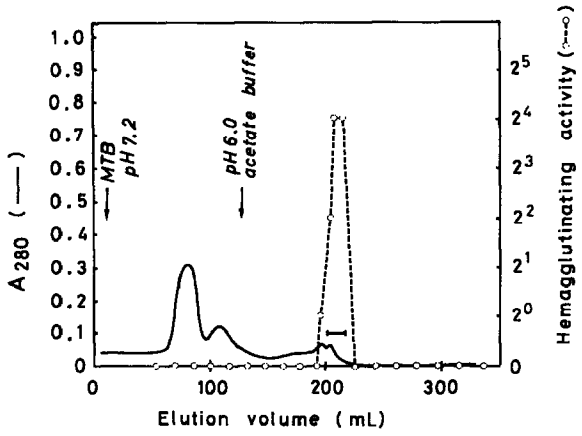

Fig. 2. DEAE-Sephadex column chromatography of crude bovine kidney lectin. Hemagglutinating-active fractions of bovine kidney (20 mL) obtained by gel chromatography were applied to a column (2.2 × 21 cm) of DEAE-Sephadex A-50 equilibrated against MTB. The column was washed with MTB, and bound lectin was eluted with 0.15M sodium acetate buffer (pH 6.0). Fractions of 2 mL were collected at a flow rate of 6 mL/h at 4°. The active fractions indicated in the Figure were dialyzed against water and then lyophilized.

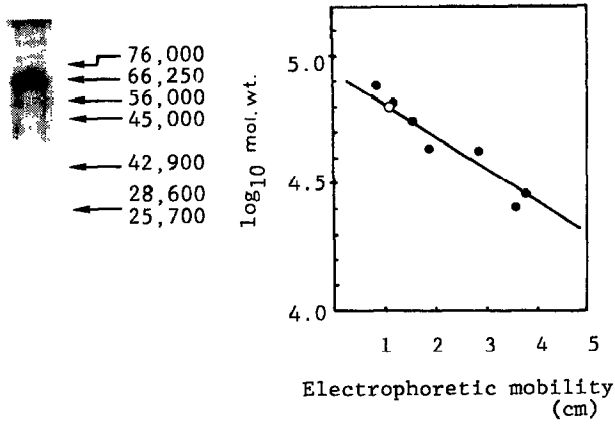

Fig. 3. SDS-Polyacrylamide gel electrophoresis of the purified bovine lectin. Left. The purified bovine lectin was dissolved in 0.0625M Tris–HCl (pH 6.8) containing 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, and 0.001% Bromophenol Blue. The sample was applied to a 12.5% polyacrylamide gel. The mobilities of molecular-weight markers are indicated by arrows in the Figure; chymotrypsinogen A (25,700), electron molecular-weight marker L (BDH Chem. Ltd.) (28,600 and 42,900), ovalbumin (45,000), electron molecular weight marker H (56,000), bovine serum albumin (66,250), and ovotransferrin (76,000).

common presence of lectin in mammalian kidneys suggests its possible involvement in the reabsorption of glycoproteins from the urine in the renal tubules. Moreover, the interaction between Tamm–Horsfall glycoprotein and the renal lectin is pathologically interesting, since Tamm–Horsfall glycoprotein is suggested to be involved in urinary stone formation[32] and many other renal diseases[30,33–35]. Complete

TABLE IV

AMINO ACID COMPOSITION OF BOVINE KIDNEY LECTIN

Amino acid	*mol %*	*Amino acid*	*mol %*
Asp	8.79	Met	—
Thr	4.89	Ile	3.33
Ser	15.71	Leu	5.33
Glu	14.20	Tyr	0.99
Pro	3.63	Phe	3.06
Gly	17.04	Lys	4.54
Ala	8.40	His	1.01
½ Cys	—	Arg	2.47
Val	6.59		

purification, characterization, and localization in the kidney of all of the lectins and also their inhibitors are necessary to elucidate the roles of these molecules in the kidney.

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