Studies on Chicken Liver Lectin

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Abstract

A lectin highly reactive with dermatan sulfate (DS-lectin) was purified from adult chicken liver using new affinity adsorbents which were prepared by immobilizing dermatan sulfate or heparin at the reducing ends on hydrazino-Toyopearl. The DS-lectin behaved as a single protein with a molecular weight of approx. 180,000 by gel chromatography, but could be further dissociated into three proteins with apparent molecular weights of 40,000, 47,000 and 87,000 on SDS polyacrylamide gel electrophoresis.

On excitation at 280 nm, the DS-lectin emitted fluorescence centered at 336 nm, which was attributable to tryptophan residues and could be quenched by the addition of specific saccharides. The association constants of the DS-lectin with specific saccharides were calculated from changes in intensities of fluorescence-difference spectra induced by the saccharides. Dermatan sulfate and protuberic acid, which is composed of L-iduronic acid and D-glucuronic acid (1 : 2), had the highest association constants among polysaccharides tested. Partially *N*-desulfated heparin had a higher association constant than that of native heparin while dextran sulfate had no affinity. D-Glucuronic acid and *N*-acetylneuraminic acid induced weak but significant quenching, but not *N*-acetylgalactosamine or cellobiose. These results were essentially in agreement with those of hemagglutination inhibition tests and indicated that DS-lectin has a strong affinity for L-iduronic acid residues and probably carboxyl groups in the saccharides, while sulfate groups on the saccharides interfere with the specific interaction.

Recently, a number of lectins have been isolated from animal origin in addition to plants and microorganisms[1-3]. The specificities of these lectins to glycoproteins have been extensively investigated, while only a little is known about the specificities against glycosaminoglycans. Barondes and coworkers have discovered heparin-inhibitable lectins in chicken liver[4], embryonic chick muscle[5] and young rat lung[6] and reported on subcellular localization and association with DNA. A cell surface agglutinin of a human teratocarcinoma cell line[7] and a lectin from the hemolymph of silkworm[8] were also inhibited by heparin. Fibronectin, another class of heparin binding substance, is a multifunctional glycoprotein, and is distributed in extracellular matrices and basement membranes as well as in plasma and other biological fluids. Its biological functions such as cell-

cell and cell-substrate adhesion, cellular motility and differentiation and wound healing have been attributed to its binding ability with various biomolecules, including heparin and other glycosaminoglycans. However, the detailed specificities and biological functions of heparin binding lectins have yet to be established.

In previous studies, we have found that plant lectins, potato lectin and wheat germ agglutinin bind specifically with sulfated glycosaminoglycans, such as keratan sulfate and chitin sulfate, which have 3-hydroxy free *N*-acetyl-6-*O*-sulfo-β-D-glucosaminyl residues as constituents[9,10]. The binding parameters of these specific interactions were successfully obtained by ultraviolet difference spectroscopy[9] and fluorescence difference spectroscopy[10]. In this report, a lectin highly reactive with DS[1] was purified from adult chicken liver by new affinity adsorbents and its binding specificity was extensively studied by a quantitative method, fluorescence difference spectroscopy.

Materials and Methods

Materials—DS (C/O, 0.59; sulfate, 16.2%) and hyaluronic acid from rat skin were prepared as described previously[11]. DS from rooster comb (C/O, 0.46; sulfate, 14.7%) was obtained from Seikagaku Kogyo (Tokyo, Japan), and further purified by alcohol fractionation before use. Hep (C/O, 3.83; sulfate, 30.3%) was purchased from Wako Pure Chemicals Ind. (Osaka, Japan). Protuberic acid (L-iduronic acid/D-glucuronic acid≒1 : 2; MW 100,000) from *Kobayasia nipponica* was generous gift from Prof. T. Miyazaki (Tokyo College of Pharmacy)[12]. Alginic acid, colominic acid and heparan sulfate (1.25 M NaCl fraction from bovine kidney, total sulfate 16.0%; *N*-sulfate, 7.29%) was obtained from Seikagaku Kogyo. Chitin sulfate was prepared from the test of tunicate[13]. Partially *N*-desulfated Hep was prepared by the treatment with dimethylsulfoxide-5% H_2O for 1.5 h at 50°C[14] (total sulfate, 22%; *N*-sulfate, 8%). Dextran sulfate (S, 17.1%; MW, 500,000) was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). ChS-A from whale cartilage was prepared as described previously[15].

DNase I from bovine pancreas and DNA from bovine thymus were

[1] The abbreviations used are: DS, dermatan sulfate; Hep, heparin; ChS-A, chondroitin sulfate A; C/O, the ratio of glucuronic acid by the carbazole reaction to glucuronic acid by the orcinol reaction; MEPB, 75 mM Na_2HPO_4/KH_2PO_4 (pH 7.2), 75 mM NaCl, 4 mM β-mercaptoethanol, 2 mM EDTA containing 1 M NaCl and 0.1 M lactose; MTB, 10 mM Tris/HCl (pH 8.6), 0.1 M NaCl, 4 mM β-mercaptoethanol; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; MW, molecular weight; K_a, association constant (M^{-1}); GAG, glycosaminoglycan.

purchased from Sigma (St. Louis, USA), and neuraminidase from *Arthrobacter ureafaciens* from Nakarai Chemicals (Kyoto, Japan). Molecular weight standard kits were purchased from Serva (Heidelberg, Germany) and RNA polymerase B was from Seikagaku Kogyo. Toyopearl HW-65 diol-type and Toyopearl HW-55, -65 and -75 (fine) were from Toyo Soda Kogyo (Tokyo, Japan).

Lectin Preparation—From the livers of adult chicken, extraction was performed according to the method of Ceri et al.[4], except for using 3 parts of MEPB. After centrifugation at 30,000×g for 2 h, 3 ml of the supernatant was applied to a Toyopearl HW-55, -65 or -75 column (2.8×18.5 cm) which had been equilibrated with MTB and the column was eluted with the same buffer. Fractions containing lectin activity were pooled and applied directly to an affinity column which was prepared by coupling DS from rat skin or Hep to 2-hydroxy-3-hydrazinopropyl-Toyopearl HW-65 diol-type[16] in the presence of $NaCNBH_3$[17]. After washing with MTB, containing 0.1 M NaCl, elution was carried out by 1 M NaCl in MTB. For large scale purification in a Hep-column, the elution was performed by increasing the concentration of NaCl as 0.25 M, 0.5 M, 0.75 M, 1 M and 3 M.

Gel Electrophoresis—Gel electrophoresis in the presence of SDS was performed according to the method of Laemmli[18] using a 3% acrylamide stacking gel and 12.5% acrylamide separating gel. Sample solutions were boiled for 10 min at 100°C before electrophoresis. Gel electrophoresis in the absence of SDS and β-mercaptoethanol was performed under the same conditions using a 7% acrylamide separating gel. Proteins were stained with Coomassie brilliant blue.

Gel Filtration—To estimate the molecular weight of the lectin, the purified lectin (60 μg) was loaded onto a column of Sepharose 2B (2.6×35 cm) which had been equilibrated with MTB. The following were used as standards: ferritin, catalase, concanavalin A and phenol red.

Chemical Analysis—2-Deoxyribose was determined by the diphenylamine assay[19]. Neutral sugars were determined by the anthrone method[20]. The amount of Hep or DS immobilized on the gel was determined by the content of hexosamine in the gel. After hydrolysis of 30 mg of suction dried Hep- or DS-Toyopearl with 1 ml of 3 M HCl at 100°C for 15 h in a sealed tube, hexosamine was determined by the Blix-Gardell method[21]. Protein was determined by the method of Lowry et al.[22]. Amino acid analyses were carried out with a Hitachi 835 amino acid analyzer after hydrolysis in 6 M HCl at 110°C in a vacuum-sealed tube for 24, 48 and 72 h. Tryptophan was estimated by the spectrophotometric method[23].

Enzyme Treatment—Trypsinized and glutaraldehyde-fixed sheep erythrocytes (10^8 cells per ml) were incubated with 1 unit of neuraminidase

overnight at 37°C in 1 ml of phosphate-buffered saline (pH 6.8) with gentle shaking[24]. The treated cells were washed three times with MTB before performing a hemagglutination assay. DNase I treatment of the fraction eluted from Hep-Toyopearl was done by the method of Kunitz[25] and the depolymerization of DNA was monitored based upon the increased UV absorption at 260 nm[25].

Hemagglutination Assays—Lectin activity was assayed with trypsinized and glutaraldehyde-treated rabbit or sheep erythrocytes that had been stored at 4°C for more than three months after preparation[5]. Hemagglutinating activity was determined using serial 2-fold dilutions of extract or purified lectin in microtiter V-plates (Cooke Engineering). 25 μl of 4% erythrocyte suspension in MTB and 25 μl of sample solution were mixed in each well and incubated for 1 h at room temperature. The same activity was observed when MEPB was used as a solvent instead of MTB. Titer is taken as the reciprocal of highest dilution of the sample causing hemagglutination and the specific activity is expressed as titer divided by the protein concentration (mg/ml) of the sample.

The inhibitory activity of the haptens was determined by adding an erythrocyte suspension, after the incubation of the lectin solution (titer =4) with the serial 2-fold dilutions of the hapten solutions for 2 h at room temperature. The lowest concentration that reduced the hemagglutination titer by one step is referred to as the concentration of hapten inhibitor that gave 50% inhibition of lectin activity.

Fluorescence Difference Spectroscopy—Fluorescence data for the purified lectin were obtained with a Hitachi type 650-60 fluorescence spectrophotometer equipped with a chart recorder. Correction of emission spectra with rhodamine B and measurements of fluorescence difference spectra were performed with a microcomputer interfaced to the fluorescence spectrophotometer. The purified lectin solution in MTB (A_{280}=0.1) and various saccharide solutions dissolved in the same buffer (0.3-40 mg/ml) were used. All measurements were carried out at room temperature, from 300 nm to 400 nm, at a scan speed of 180 nm/min with excitation at 280 nm. For determination of the association constants, the peak intensities of difference spectra were determined as a function of saccharide concentration. Corrections were made for the fluorescence of polysaccharides as necessary.

Results

Purification of the Lectin—When crude extract was subjected to gel filtration on Toyopearl HW-55, -65 or -75 columns, hemagglutinating

activities were excluded from all columns and a Toyopearl HW-55 column was used thereafter to obtain the lectin fraction. The affinity chromatogram of the lectin fraction obtained using the DS-column (0.9 mg DS/g wet gel) is shown in Fig. 1. The large peak having hemagglutinating activity was eluted with 1 M NaCl. A similar chromatogram was obtained using a Hep-column (1.1 mg Hep/g wet gel). The 1 M NaCl fractions from the DS- and Hep-columns gave identical results in hemagglutination inhibition studies, and fluorescence emission spectra, suggesting that the same lectin was being obtained by the two columns. However, the 1 M NaCl fractions showed numerous bands on SDS-PAGE. To separate the lectin from other proteins, stepwise elution with lower concentrations of NaCl was performed in a large Hep-column (Fig. 2), rather than the more expensive DS-columns. High hemagglutinating activities, corresponding to high absorbance at 280 nm, were observed in fractions eluted with 0.25 M, 0.5 M and 0.75 M NaCl, whereas those of the fractions eluted with 1 M, 3 M NaCl and 3 M KSCN were low.

The results of a typical preparation of the lectin are summarized in Table I. Although the 0.5 M and 0.75 M NaCl fractions showed rather higher hemagglutinating activity, compared to that of the 0.25 M NaCl fraction, these fractions showed UV absorption spectra with a maximum near 260 nm. The high contents of 2-deoxyribose in these fractions were confirmed by the diphenylamine assay and DNase treatment of the 0.5 M NaCl fraction causing an increase of absorbance at 260 nm (data not shown). The results indicated that the 0.5 M and 0.75 M NaCl fractions contained considerable amounts of DNA. However, DNA solution (>140 μg/ml as

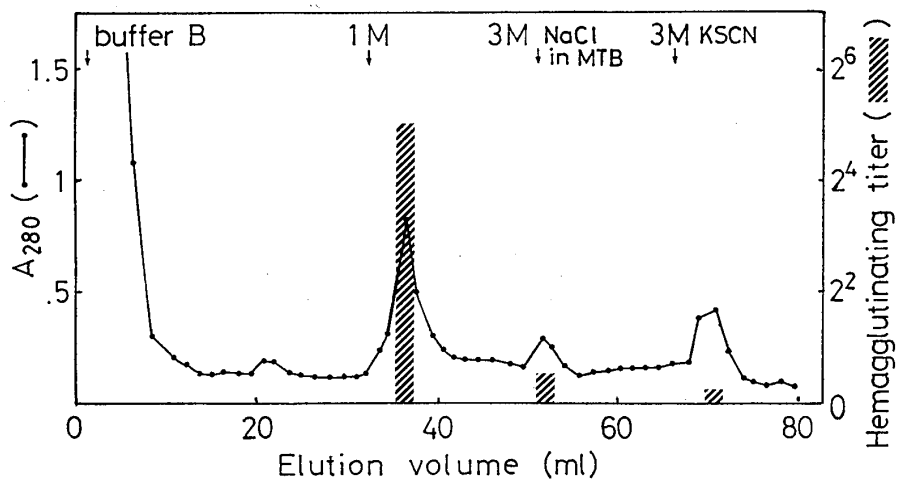

Fig. 1. *Affinity chromatography of crude extract on DS-Toyopearl.* The crude extract from 15 g of fresh liver was applied directly to a DS-column (0.85×4.0 cm). The elution was performed successively with 1 M NaCl, 3 M NaCl and 3 M KSCN.

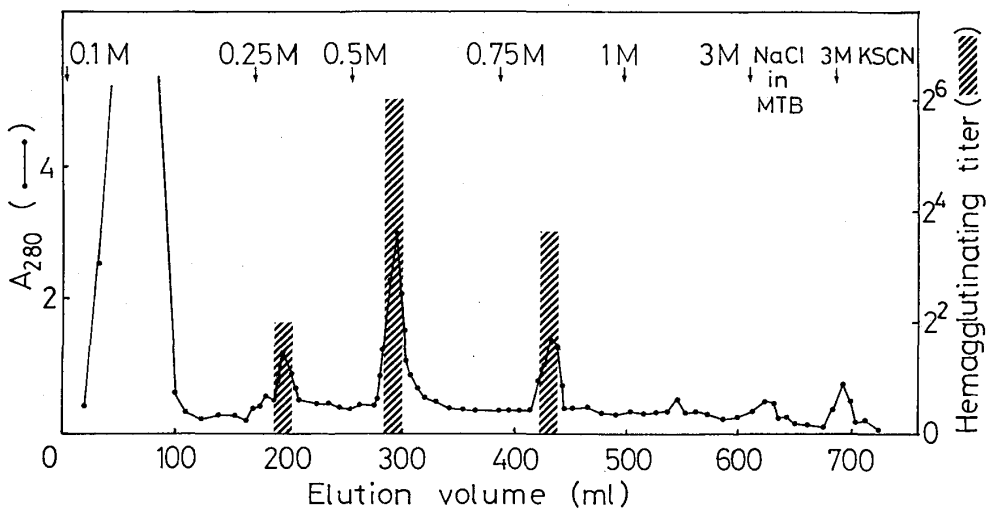

Fig. 2. *Affinity chromatography of crude lectin on Hep-Toyopearl.* The excluded fraction on Toyopearl HW-55 (crude lectin), which was extracted from 15 g of liver, was applied to a Hep-column (1.8×11.5 cm) and eluted with increasing concentrations of NaCl. 3 ml fractions were collected and assayed for lectin activity.

Table I. Purification of DS-lectin.
The purification procedure is described in the text. Similar data were obtained in a number of independent purifications.

Fraction	Hemagglutinating activity $(mg/ml)^{-1}$	Protein (mg)	% Activity recovered	UV_{max} (nm)	2-Deoxyribose / Protein
Extract	12	617	100	—	—
After Gel Chromatography	12	373	60	—	—
After affinity Chromatography					
0.25 M NaCl	8.2	11.7	1.3	279	0.04
0.5 M NaCl	31	8.5	3.6	262	0.26
0.75 M NaCl	73	3.8	3.7	258	0.23

2-deoxyribose) itself caused hemagglutination of the trypsinized glutaraldehyde-fixed erythrocytes. Also the hemagglutinating activity of these fractions was diminished after DNase treatment and the addition of DNA to the 0.25 M NaCl fraction enhanced hemagglutinating activity and its stability (although it lost the activity within several days when stored at 4°C). Furthermore, fluorescence intensities of the 0.5 M and 0.75 M fractions were very weak compared to that of the 0.25 M NaCl fraction and it was impossible to evaluate their specificity by fluorescence difference spectroscopy. Therefore the 0.25 M NaCl fraction was used as a purified lectin fraction despite its low activity and low recovery.

Gel filtration of the 0.25 M NaCl fraction in Sepharose 2B gave a single symmetric peak detected by protein assay (Fig. 3). The elution

position of the protein corresponded to that of a globular protein of a molecular weight of 180,000. The 0.25 M NaCl fraction migrated as a single but broad band on PAGE, in the absence of SDS, and gave three bands corresponding to molecular weights of 40,000, 47,000 and 87,000 in the presence of SDS as shown in Fig. 4. However, the 1 M NaCl fraction, which varied in recovery for each preparation, was similar to the Hep-lectin[4] with regard to the SDS-PAGE pattern, and in the association with DNA[6], as previously reported by Barondes and coworkers.

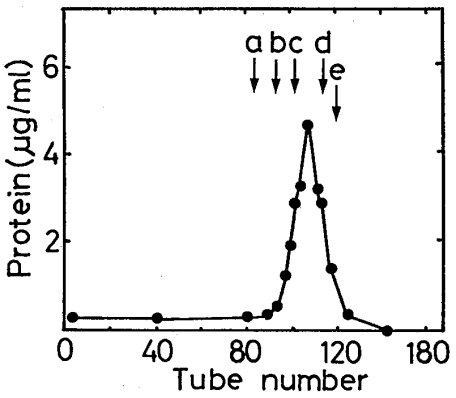

Fig. 3. *Sepharose 2B gel filtration of purified lectin.* Fractions of 2 ml were collected at a flow rate of 12 ml/h and assayed for protein. a, blue dextran 2000; b, ferritin; c, catalase; d, concanavalin A; e, phenol red.

Chemical Composition—The amino acid composition of the 0.25 M NaCl fraction is shown in Table II. The main amino acids were glycine, serine, glutamic acid, alanine and aspartic acid. This lectin contained 1 mol% of tryptophan and no half cystine. The neutral sugar content was 3% as D-galactose.

Hemagglutination Inhibition Activity—As shown in Table III, about

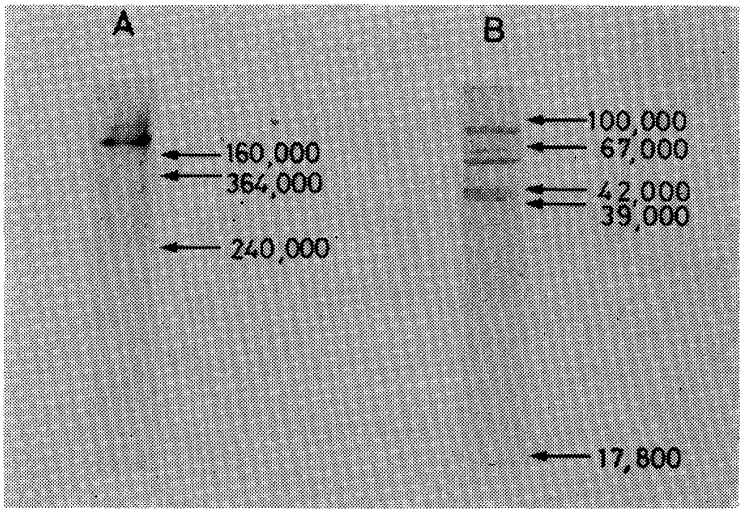

Fig. 4. *Polyacrylamide gel electrophoresis of the purified lectin.* A, The purified lectin dissolved in 62.5 mM Tris/HCl (pH 6.8) containing 10% glycerol (about 6 μg/6 μl) was applied to a 7% acrylamide gel. Electrophoresis was performed in the absence of SDS and β-mercaptoethanol for 4 h at 4°C. B, Electrophoresis in the presence of 2% SDS and 5% β-mercaptoethanol was performed in a 12.5% gel for 3 h at 4°C. The following proteins were used as standards: myoglobin (17,800), RNA polymerase B (39,000, 42,000 and 100,000), bovine serum albumin (67,000), aldolase (160,000), catalase (240,000) and ferritin (364,000).

Table II. Amino acid composition of DS-lectin.
Hydrolysis and amino acid analysis of the lectin were performed as described under the text. The result is expressed as mol% of amino acid residues.

amino acid	mol %
Aspartic acid	8.7
Threonine	5.9
Serine	6.4
Glutamic acid	10.5
Proline	5.5
Glycine	8.3
Alanine	9.4
Valine	7.2
Methionine	2.2
Isoleucine	6.1
Leucine	9.0
Tyrosine	3.0
Phenylanine	3.1
Lysine	7.4
Histidine	1.9
Arginine	4.5
Tryptophan	1.0

Table III. Inhibition of lectin activity by GAGs and monosaccharides.
Hemagglutination activity of lectin purified by affinity chromatography was determined in the presence of a range of concentrations of the saccharides. The data represent the average results from three assays.

GAGs and monosaccharides	Concentration of GAGs and monosaccharides yielding 50% inhibition of lectin activity (mg/ml)	(M)
Heparin	0.52	1.7×10^{-5}
Dermatan sulfate	0.95	4.0×10^{-5}
Chondroitin sulfate A	(>11.7)	
N-Acetyl-D-glucosamine	>5.1	$>2.3\times10^{-2}$
N-Acetyl-D-galactosamine	>8.3	$>3.7\times10^{-2}$
D-Glucuronic acid	>8.8	$>4.5\times10^{-2}$

10^{-5} M of Hep and DS produced 50% inhibition. However, none of the monosaccharides had inhibitory activity at the concentrations tested. On the contrary, ChS-A enhanced the hemagglutinating activity of the lectin.

Fluorescence Spectroscopic Studies—On excitation at 280 nm, the 0.25 M NaCl fraction (DS-lectin) showed a fluorescence emission spectrum with a maximum at 336 nm, which was attributable to tryptophan residues. The fluorescence emission intensity was markedly quenched by the addition of DS (<1 mM) and other acidic polysaccharides as shown in Fig. 5. These

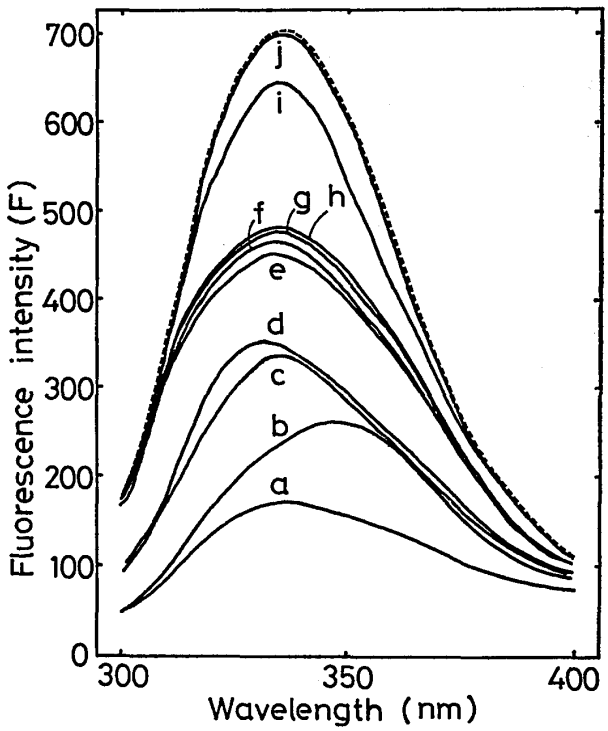

Fig. 5. *Fluorescence emission spectra of DS-lectin in the presence of various saccharides.* ·····, Spectrum of DS-lectin alone; ———, spectra in the presence of saccharides. The final concentrations of various sugars are indicated in the parentheses. Corrections were made for the fluorescence of polysaccharides. a, DS (0.11 mM); b, Hep (0.77 mM); c, heparan sulfate (0.53 mM); d, protuberic acid (0.03 mM); e, *N*-acetyl-D-neuraminic acid (130 mM); f, D-glucuronic acid (130 mM); g, 2-deoxy-D-ribose (130 mM); h, cellobiose (140 mM); i, *N*-acetyl-D-galactosamine (235 mM); j, dextran sulfate (0.06 mM), D-glucose, D-galactose, L-fucose and D-mannose (all 250–300 mM).

changes were not observed on addition of *N*-acetyl-D-galactosamine or neutral sugars (>200 mM) and it was therefore assumed that the quenching was due to a specific interaction between DS and this lectin. The fluorescence-quenching depended on the DS concentration and plots of the intensity difference (ΔF) in the difference spectra against the DS concentration were hyperbolic, as shown in Fig. 6. Plots according to the equation, $\log \Delta F/(\Delta F_{max}-\Delta F)=\log[DS]+\log K_a$ were linear with a slope of 1 ± 0.05, as shown in Fig. 7, where K_a is the association constant of the lectin-DS complex, [DS] is the concentration of DS and ΔF_{max} is the maximum intensity difference obtained when all the lectin is in the form of a complex with DS. Estimating the molecular weight of the polysaccharides from their reducing values, the K_a values of this lectin with specific saccharides were calculated as shown in Table IV.

Acidic polysaccharides had generally high K_a values, and DS and protuberic acid, both of which contain relatively large amounts of L-

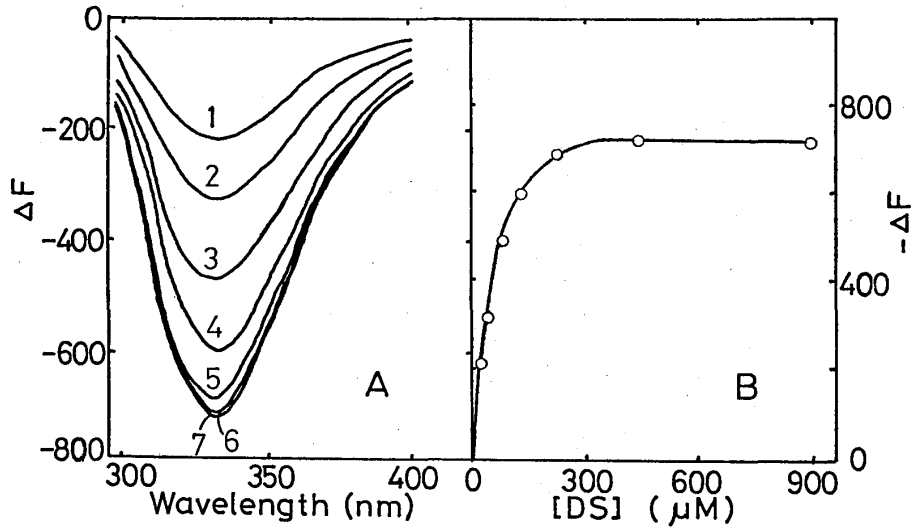

Fig. 6. *Fluorescence-difference spectra of DS-lectin induced by DS (A) and the effect of DS concentration on the difference spectra (B).* The concentrations of DS were (1) 14 μM, (2) 28 μM, (3) 56 μM, (4) 112 μM, (5) 224 μM, (6) 448 μM and (7) 886 μM.

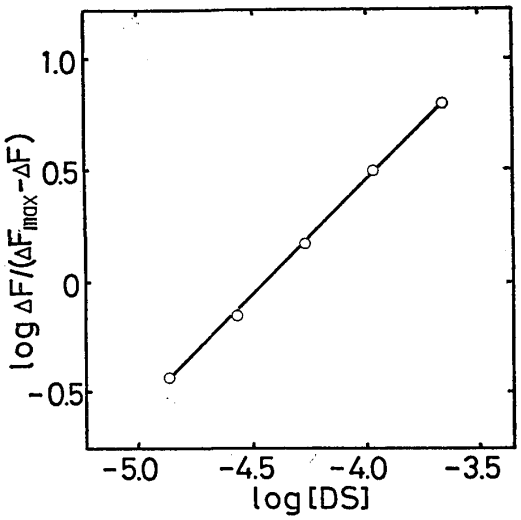

Fig. 7. *Plots of* $\Delta F/(\Delta F_{max}-\Delta F)$ *versus log* [*DS*].

iduronic acid, had the highest K_a values among saccharides tested. Hyaluronic acid has a rather high K_a value among acidic polysaccharides containing uronic acid other than iduronic acid. This may be because hyaluronic acid having the highest molecular weight exhibited the largest multivalent effect. Among monosaccharides and oligosaccharides tested, *N*-acetyl-D-neuraminic acid, and D-glucuronic acid induced weak but significant quenching, but not most neutral sugars including cellobiose and *N*-acetyl-D-galactosamine. These results indicate that the lectin can interact with the sugar residues having carboxyl groups, and has the highest affinity to L-iduronic acid residues in the polysaccharides.

Dextran sulfate did not interact with the lectin, furthermore heparin and heparan sulfate, which contain *N*-sulfated D-glucosamine and sulfated L-iduronic acid residues, had relatively low K_a values. Also partial *N*-desulfation of heparin resulted in an increase of the K_a value. These results suggest that sulfate groups in the polysaccharides are not responsible for, but rather interfere with, the specific binding to the lectin.

Table IV. Association constants (K_a) of DS-lectin with various saccharides. K_a were obtained by plotting the data of fluorescence-difference spectra induced by each saccharide as shown in Fig. 7.

Saccharides	MW	K_a (M^{-1})
Dermatan sulfate	44,000	4.2×10^4
Protuberic acid	100,000	3.2×10^4
Hyaluronic acid	104,000	1.9×10^4
Alginic acid	11,000	5.0×10^3
Colominic acid	11,000	3.5×10^3
Heparin	30,000	1.8×10^3
N-Desulfated heparin	15,000	3.1×10^3
Heparan sulfate	18,000	9.8×10^2
N-Acetyl-D-neuraminic acid	309	9×10^1
D-Glucuronic acid	194	6×10^1
2-Deoxy-D-ribose	134	4×10^0
Cellobiose	342	3×10^0
Dextran sulfate	500,000	0
D-Galactose, D-Glucose, D-Mannose	180	0
L-Fucose	154	0
N-Acetyl-D-galactosamine	221	0
D-Galactosamine	179	0

Discussion

The lectin obtained from adult chicken liver by affinity chromatography on a Hep- or DS-column was found to be highly reactive for DS by fluorescence spectroscopy and hemagglutination inhibition assays. Such a lectin has not hitherto been reported, and the name 'DS-lectin' is proposed.

DS-lectin behaved as a very large molecular weight aggregate on gel filtration, just after extraction, and the hemagglutinating activity could be adsorbed on a new affinity adsorbent (Hep- or DS-column). The 0.25 M NaCl fraction from the Hep-column was almost entirely free of DNA and gave a single protein peak corresponding to a molecular weight of 180,000 on gel filtration and was dissociated into three bands with apparent molecular weights of 40,000, 47,000 and 87,000 on SDS-PAGE. From these results, DS-lectin seemed to be composed of three different size of subunits or of two identical 87,000 monomers which consist of 40,000 and 47,000 subunits. The contaminating DNA appeared to enhance the specific activity and stability of the lectin, so the low recovery of the activity in the 0.25 M NaCl fraction would be due to the low content of DNA.

On interaction with specific saccharides, DS-lectin induced a fluorescence difference spectrum due to the tryptophan residues. This permitted quantitative and reproducible analysis of the interaction, whereas the

hemagglutination inhibition test gave only an approximate indication of the specificity of the lectin. Comparing the association constants of DS-lectin with various saccharides, it was shown that the lectin could interact with saccharides which have carboxyl groups. The highest affinity found was for L-iduronic acid residues. Dextran sulfate failed to induce any change in the fluorescence spectrum of DS-lectin. This rules out the possibility that the fluorescence changes observed were due to non-specific electrostatic interactions. However, free tryptophan showed a fluorescence emission spectrum with a maximum of 355 nm under the same conditions: the maximum intensity was reduced to 63% by the addition of Hep (11.7 mg/ml) and the shape of the difference spectrum of tryptophan caused by Hep was the same as that of DS-lectin. This is strong evidence for DS-lectin having tryptophan residues at or near the sugar binding sites and having an involvement with the specific interaction.

Quenching of the fluorescence intensity of DS-lectin was induced by chitin sulfate and also weakly by $(GlcNAc)_4$. The presence of GlcNAc-specific lectin in chicken liver has been reported by Kawasaki and Ashwell[26], but DS-lectin is distinct from this lectin in molecular weight and no contamination with this lectin was observed by SDS-PAGE and gel filtration.

The hemagglutinating activity of DS-lectin and stability were increased by DNA. However, the enhancement effect was not found with 2-deoxyribose and was therefore not due to any sugar-lectin specific interactions but was rather the result of indirect effects, such as aggregation or conformational changes of the lectin, triggered by electrostatic interactions with the DNA. An enhancement of the hemagglutinating activity of DS-lectin was also observed on addition of ChS-A, which might be due to a similar effect as with DNA.

The nature of the sugar residues which are recognized by DS-lectin on the surface of trypsinized glutaraldehyde-fixed erythrocytes is of some interest. The hemagglutinability was decreased by neuraminidase-treatment of the sheep erythrocytes from a titer of 2^5 to 2^2. Even though the hemagglutinability was not completely abolished by this treatment, the finding that this lectin has some affinity toward *N*-acetyl-D-neuraminic acid, as shown by fluorescence spectroscopy, suggests that the sialic acid residues on the cell surface of erythrocytes are involved in at least a part of the binding to DS-lectin. The Hep-lectin found by Ceri et al. had subunits with molecular weights of 16,000 and 13,000, as shown by SDS-PAGE and affinity toward *N*-acetyl-D-galactosamine and cellobiose[4], but could not agglutinate formalinized sheep erythrocytes[27]. In these respects, DS-lectin is different from Hep-lectin, although both lectins were obtained by affinity chromatography on a Hep-column. In the results of the present

work Hep was immobilized *via* its reducing end, while the Hep-Sepharose of Ceri et al. was prepared by the CNBr method[28]. The former type of immobilized Hep maintains high affinity to Hep binding proteins, as clearly shown for antithrombin-III[17]. There were indications that components separated by SDS-PAGE from the 1 M NaCl fraction of this work were similar to those reported for Hep-lectin, although it was impossible to study precise binding specificities from fluorescence emission spectra, probably because of the high content of DNA. From these results, it was assumed that DS-lectin and Hep-lectin were adsorbed on our Hep-column and were eluted with 0.25 M and 1 M NaCl, respectively. In the results of Ceri et al,, only Hep-lectin was adsorbed on the column. That may have been because DS-lectin has a lower affinity to Hep-lectin and/or requires an intact structure of Hep in order to bind: the difference between the modes of Hep immobilization may be critical for binding with DS-lectin.

Lectin like substances seem to be abundant in animal liver and many other hepatic lectins and sugar binding proteins had been reported[26,29-31]. The presence of similar but distinct lectins such as DS-lectin and Hep-lectin is therefore not surprising. Preliminary experiments indicated the presence of lectins having similar specificity to DS-lectin not only in the liver but also in lung and heart of rats and bovine kidney. It is unclear whether these are the same lectin as the DS-lectin, but it is almost certain that the GAG-binding lectins are widely distributed in animal tissues. It is likely that much interest will be concerned with the physiological roles of these different lectins. Almost all of these lectins are extracted without detergent and may be located on the cell surface membrane. They may participate in cellular adhesion or formation of extracellular matrices such as fibronectin[32], by binding with the intercellular or cell surface GAG. However, DS-lectin differs from fibronectin in molecular weight, amino acid composition and binding properties to DS. Another possible role of GAG-specific lectin may be as a cell surface receptor for the metabolism of GAG[30] or for the functional expression of different types of GAG such as a modulator of cellular interaction[33]. Regarding the DS-lectin in the chicken liver, the association constants for GAG are relatively low ($10^4\ M^{-1}$) and reversibly forms soluble complexes under physiological conditions. There is therefore some possibility of a regulatory function *in vivo* for DS-lectin, involving the reversible interaction with GAG although regarding this point further studies will be required.

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