Comparative Studies of Carbohydrate-Binding Proteins from *Xenopus laevis* Skin and Eggs. Sugar-Binding Specificities and Affinity Purification[1)]

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Salt and detergent extracts of acetone-dried powder of *Xenopus laevis* skin and eggs were fractionated on sugar–Sepharose columns, to which lactose, melibiose, galactose, rhamnose and mannose had been covalently linked, by successive elution with chelating reagent and specific sugars, resulting in separation of the different Ca^{2+}-dependent and Ca^{2+}-independent carbohydrate-binding proteins. The skin of *X. laevis* contains a salt-extractable Ca^{2+}-dependent lactose-binding lectin of 30 kilodalton (kDa) and the eggs a similar lectin of 43 kDa, but they both lack Ca^{2+}-dependent galactose-binding lectins. The 30 kDa lactose-binding lectin which agglutinates human A erythrocytes was isolated by successive affinity chromatography on two linked sugar–Sepharose columns, *i.e.*, a galactose–Sepharose–lactose–Sepharose (GL) column system. Since the 30 kDa lectin was not recovered in the Ca^{2+}-dependent lactose-binding protein fraction from the GL column system under the dithiothreitol (DTT)-free conditions, it was concluded that the lectin requires the presence of DTT and calcium for binding to the lactose–Sepharose column.

Keywords lectin; *Xenopus laevis*; sodium dodecyl sulfate-polyacrylamide gel electrophoresis; sugar–Sepharose column; affinity chromatography; calcium(II)-dependent sugar-binding protein; calcium(II)-independent sugar-binding protein

Lectins are divalent or multivalent carbohydrate-binding proteins with the ability to agglutinate erythrocytes, tumor cells and bacteria. Because of their ability to recognize sugar residues on cell surface receptors, they have been widely used in the purification of polysaccharides and glycoproteins and in biological applications, including targeting of toxins to tumor cells and subcellular localization of glycoconjugates.[2,3)]

Lectins may be isolated by conventional protein-purification techniques, affinity chromatography, or a combination thereof.[4,5)] Affinity chromatography has been applied to lectin purification, as follows: the carbohydrate ligand with which the lectin interacts is insolubilized, the lectin is adsorbed, and displacement of bound lectin is accomplished by elution, either with a sugar that competes for lectin sites with the specific adsorbent, or by altering the nature of the eluant (lowering the pH, increasing the ionic strength, or adding denaturants).

Galactose-specific receptors (lectins) which bind to asialoglycoproteins have been isolated from rat,[6)] rabbit[7)] and human liver.[8)] Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) has revealed multiple polypeptide species in both rat and rabbit preparations.[6,7)] Halberg *et al.*[9)] reported that major and minor forms of the rat liver asialoglycoprotein receptor (rat hepatic lectin) each have specific galactose-binding activity and that the major and minor polypeptide species each form homooligomers.

In this study, we have analyzed sugar-binding proteins in *Xenopus laevis* skin and eggs using PAGE in the presence of SDS and have separated two salt-extractable Ca^{2+}-dependent lactose-binding lectins from *X. laevis* skin and eggs by means of successive affinity chromatographies on two linked sugar–Sepharose (galactose–Sepharose and lactose–Sepharose) columns. The results indicate that multiple species of sugar-binding proteins are present in the skin and the eggs and that different forms of these proteins may serve unique functions in each organ.

Materials and Methods

Materials Saccharides were obtained from Fluka and Nacalai Tesque (Kyoto, Japan); Sephadex G-75 and Sepharose 6B were from Pharmacia-LKB; divinyl sulfone (DVS), dithiothreitol (DTT) and phenylmethanesulfonyl fluoride (PMSF) were purchased from Aldrich, Nacalai Tesque, and Sigma, respectively.

The hemagglutination active fraction from *X. laevis* skin was prepared by Sephadex G-75 gel filtration as described previously,[10)] and termed "S1 fraction." The tumor cell agglutination active fraction from *X. laevis* eggs was prepared by Sephadex G-75 gel filtration as described previously,[11)] and termed "E1 fraction."

Preparations of Resins for Affinity Chromatography Lactose, melibiose, D-galactose, D-mannose and L-rhamnose were coupled to Sepharose 6B after activation with DVS according to the method described by Fornstedt and Porath.[12)] Packed Sepharose 6B (200 ml) was incubated with 100 ml of 0.5 M carbonate buffer (pH 11) and 21 ml of DVS (*d*; 1.17). The mixture was kept at room temperature for 90 min with gentle shaking and washed extensively with distilled water. Then 50 ml of a 20% (w/v) sugr solution in 0.5 M carbonate buffer (pH 10) was reacted with 50 ml of the activated gel overnight under conditions similar to those described above. The gel (50 ml) was extensively washed with distilled water and then suspended in 50 ml of 0.5 M bicarbonate buffer (pH 8.5) and 1 ml of 2-mercaptoethanol for 2 h at room temperature to block any unreacted groups. Each gel was washed with distilled water, packed into a column (1.1 × 4.2 cm) and equilibrated with buffer C.

Sequential Extraction of Carbohydrate-Binding Proteins *X. laevis* skin and eggs were collected, rinsed with physiological saline, homogenized with ice-cold physiological saline and acetone (1 : 10), and dried. Each powder was extracted according to the methods described by Maynard and Baenziger[13)] and Gabius *et al.*[14)] A 20 g portion of powder was extracted twice with 360 ml of buffer A and the supernatants were combined and brought to a final concentration of 0.5% Triton X-100, 25 mM $CaCl_2$, and 1.25 M NaCl. The insoluble pellet was subsequently extracted 3 times with 310 ml of buffer B. The buffer B supernatants were combined and brought to a final concentration of 25 mM $CaCl_2$. The columns on which each extract was loaded were washed with 25 bed volumes of buffer C, eluted with buffer D to release Ca^{2+}-dependent carbohydrate-binding proteins, reequilibrated with buffer C, and then eluted with buffer E to release Ca^{2+}-independent carbohydrate-binding proteins. The eluates from each column were concentrated to a small volume, dialyzed in seamless cellulose tubing 30/32 (Visking Company) against distilled water, and lyophilized.

Successive Affinity Chromatography on Two Linked Sugar–Sepharose Columns Galactose–Sepharose and lactose–Sepharose were prepared as described above and the columns were connected in series (GL column) and equilibrated with buffer C. The "S1 fraction" (100 mg) was dissolved in 5 ml of buffer A and centrifuged for 30 min at 10000 × *g* to remove insoluble materials. The supernatant was brought to a final concentration of 0.5% Triton X-100, 25 mM $CaCl_2$, and 1.25 M NaCl, and applied to the GL column. Elution was performed first with a large amount of buffer C, and then the GL column was separated into two individual columns. Each

column was eluted with buffer D, reequilibrated with buffer C and eluted with buffer E, and the eluates were treated in the same way as described above.

On the same GL column equilibrated with buffer C′, the S1 fraction was dissolved in buffer A′ and chromatographed by the use of buffers C′, D′ and E′ instead of buffers C, D and E, respectively. The eluates were treated in the same way as described above.

A lactose–Sepharose column and a galactose–Sepharose column were connected in series (LG column), and the S1 fraction dissolved in buffer A was applied to the connected LG column. The elution was performed with buffers C, D and E, and the eluates were treated in the same way as described above.

The "E1 fraction" (100 mg) was dissolved in buffer A, treated in the same way as described above and applied to the connected GL column. Affinity chromatography was performed by the use of buffers C, D and E, and each fraction was pooled and treated in the same way as described above.

Polyacrylamide Gel Electrophoresis (PAGE) SDS-PAGE was performed in 1.5-mm thick slab gels by the method described by Laemmli[15] using a 4.5% polyacrylamide stacking gel and 10% polyacrylamide separation gel. Unless otherwise mentioned, the amount of each sample applied to the top of the gel was 50 μg protein. Non-denaturing-PAGE was performed in 7% slab gel with Tris/diethylbarbituric acid (pH 8.0) according to the method of Williams and Reisfeld.[16] Proteins were determined by the method of fluorometric assay described by Udenfriend *et al.*[17] with bovine serum albumin (BSA) as a standard. The gels were stained with Coomassie Brilliant Blue R 250 or by the silver staining method described by Merril *et al.*[18]

Hemagglutination Assay Hemagglutination assay was performed by the use of microtiter U-plates with intact and trypsin-treated rabbit erythrocytes in phosphate buffered saline (PBS) or with human erythrocytes in saline, as described previously.[19]

Agglutination Assay for MM46 Tumor Cells Agglutination activity was determined by using serial 2-fold dilutions of the sample solution in small test tubes. Each tube contained 100 μl of cell suspension (1×10^7 cells/ml) and 100 μl of sample solution and was shaken for 10 min. The agglutination was scored after the tubes had been left to stand for 30 min at room temperature.[20]

Results

Comparison of Carbohydrate-Binding Proteins from X. *laevis* Skin and Eggs SDS-PAGE patterns of carbohydrate-binding proteins from skin and eggs are shown in Fig. 1. Table I presents the molecular weights of significant protein/glycoprotein bands among many bands detected by SDS-PAGE.

When the buffer A (salt)-extractable fraction of skin (SK-A) was fractionated by the lactose–Sepharose affinity chromatography, a band at an apparent molecular weight of 30 kilodalton (kDa) was eluted by buffer D, whereas on the same affinity column chromatography, the buffer B (detergent)-extractable fraction of skin (SK-B) gave polypeptides of M.W. = 91 and 39 kDa with buffer D, and those of M.W. = 91, 39 and 35 kDa with buffer E. When buffers D- and E-eluted fractions were prepared from salt-extractable fraction of eggs (EG-A) by the same affinity chromatography as above, they gave polypeptides of M.W. = 43 and 32 kDa and M.W. = 25 kDa alone, respectively. In the case of detergent-extractable fraction of eggs (EG-B), buffer D-eluted fraction gave bands with molecular weights of 100, 77, 55 and 49 kDa and buffer E-eluted fraction, those of 63, 61, 45 and 33 kDa. The M.W. = 30 kDa band appeared characteristically in buffer D-eluted fraction of SK-A (SK-AD fraction), whereas the M.W. = 39 kDa band was detected in both buffers D- and E-eluted fractions of SK-B (SK-BD fraction and SK-BE fraction). When egg preparations were analyzed by SDS-PAGE, M.W. = 43 and 32 kDa bands were detected only in EG-AD fraction and the M.W. = 45 kDa band in both EG-BD and EG-BE fractions.

When extracts obtained by sequential extraction were chromatographed on a melibiose–Sepharose column, meli-

Table I. Carbohydrate-Binding Proteins of *Xenopus laevis* Skin and Eggs[a]

Source	Column and eluant									
	Lactose		Melibiose		Galactose		Mannose		Rhamnose	
	D	E	D	E	D	E	D	E	D	E
Skin-salt (SK-A)	30		105	45	(36)[b]	57	34	57	170	31
			61	39		46	31		160	
			27	34		43			145	
				29		36			31	
				25						
Skin-detergent (SK-B)	91	91	100	100	28	150	37	65	96	150
	39	39	91	91		96		59	40	83
		35	25	59		72		55		79
				36		58				47
				25		45				45
						37				40
										38
Egg-salt (EG-A)	43	(25)[b]	45	66	99		47	62	42	43
	32		38	39	47			47	37	37
					39			38		33
Egg-detergent (EG-B)	100	63	96	130	86	64	130	105	98	98
	77	61	66		47	47	96	45	84	65
	55	45	45		41	41	77	41	78	45
	49	33	34		38	37	28	37	71	33
	46				36	36	27		58	
	37				33				45	
									35	
									28	

a) The extraction and elution conditions are described in Materials and Methods. The lectin pattern is classified into four categories, salt-extractable (A), detergent-extractable (B), buffer D-eluted Ca^{2+}-dependent (D) and buffer E-eluted Ca^{2+}-independent (E) carbohydrate-binding proteins. The molecular weight is given in kDa. *b*) This band is obscure in the photograph in Fig. 1.

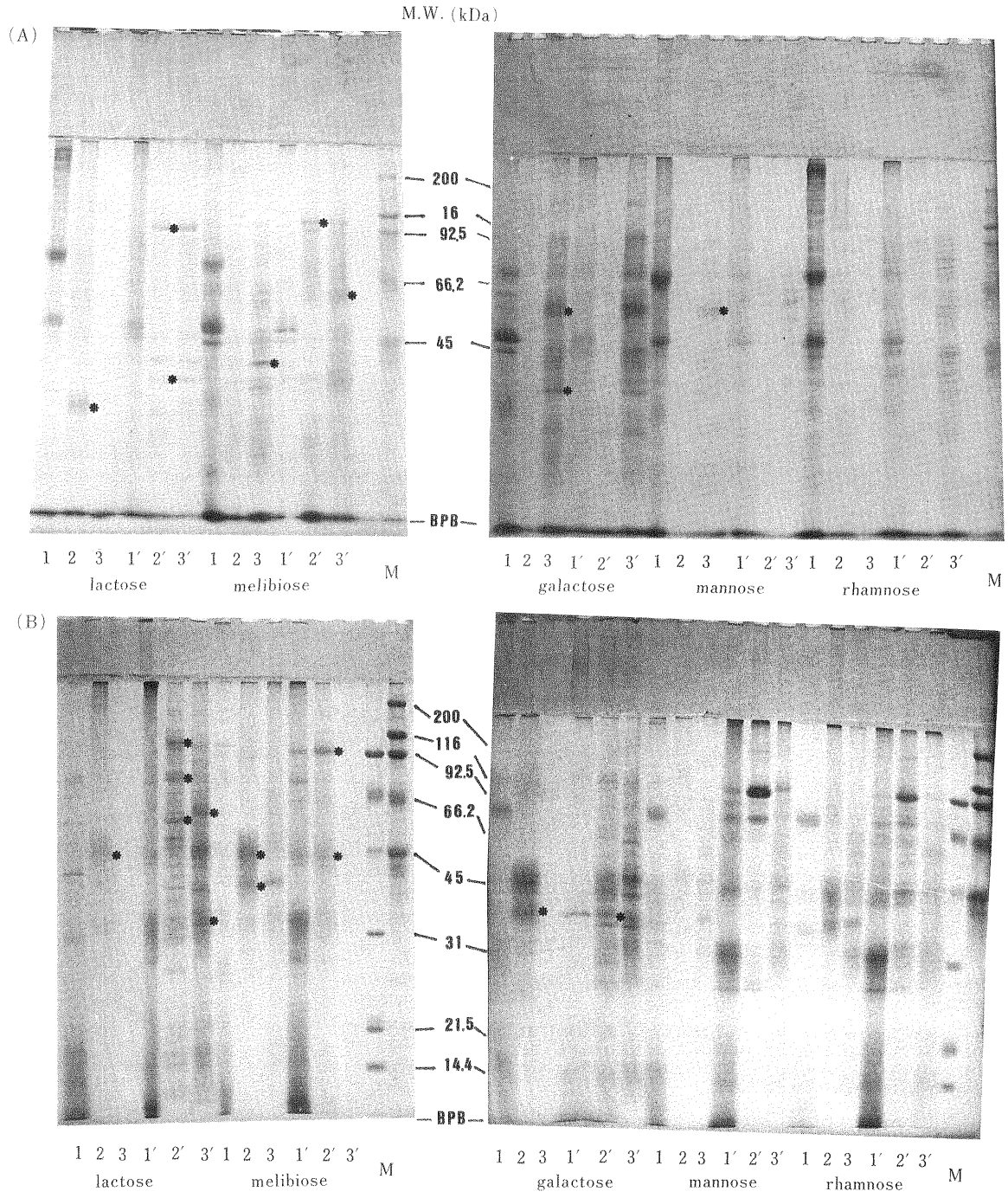

Fig. 1. SDS-PAGE of Carbohydrate-Binding Proteins from *X. laevis* Skin (A) and Eggs (B)

Electrophoresis was performed as described in the text with 10% polyacrylamide gel under reducing conditions. Migration was from top to bottom, and the bromophenol blue front is indicated (BPB). Lane 1, salt-extractable non-adsorbed protein (AC) fraction; lane 2, salt-extractable Ca^{2+}-dependent protein (AD) fraction; lane 3, salt-extractable Ca^{2+}-independent protein (AE) fraction; lane 1′, detergent-extractable non-adsorbed protein (BC) fraction; lane 2′, detergent-extractable Ca^{2+}-dependent protein (BD) fraction; lane 3′, detergent-extractable Ca^{2+}-independent protein (BE) fraction; lane M, marker proteins. Markers (from Bio-Rad) were: myosin, M.W. = 200000; β-galactosidase, M.W. = 116250; phosphorylase B, M.W. = 92500; bovine serum albumin, M.W. = 66200; ovalbumin, M.W. = 45000; carbonic anhydrase, M.W. = 31000; soybean trypsin inhibitor, M.W. = 21500. The significant protein/glycoprotein bands which may correspond to lectins reported by Bols *et al.*,[27] Roberson and Barondes[29] and Nishihara *et al.*[30] are marked with asterisks (*).

biose-binding proteins were detected as an M.W. = 39 kDa band in not only the EG-AD and EG-AE fractions but also the SK-AE fraction, and as an M.W. = 45 kDa band in EG-AD and EG-BD fractions. The galactose-binding proteins of M.W. = 36—37 kDa bands were found in SK-AD, SK-AE, SK-BE, EG-BD and EG-BE. Furthermore, M.W. = 57—58 kDa bands in SK-AE and SK-BE fractions and M.W. = 47 and 39 kDa bands in EG-AD fraction were found as to galactose-binding proteins. The mannose- and rhamnose-binding proteins are listed in Table I (see Fig. 1).

The hemagglutinating activity of carbohydrate-binding protein fractions has been determined and the results are presented in Table II. The fractions possessing strong hemagglutinating activity were as follows: SK-BD fractions from lactose– and rhamnose–Sepharose columns, SK-BE

TABLE II. Minimum Hemagglutinating Concentration of Various Carbohydrate-Binding Protein Fractions against Rabbit Erythrocytes[a]

Source	Column and eluant														
	Lactose			Melibiose			Galactose			Mannose			Rhamnose		
	C[b]	D	E	C	D	E	C	D	E	C	D	E	C	D	E
Skin-salt (SK-A)	300	200	NT	45	NT	NT	245	700	NT	2	NT	NT	180	750	40—80
Skin-detergent (SK-B)	730	2	NT	NT	NT	18—35	680	90	42	200	860	NT	100—200	3—6	1.5
Egg-salt (EG-A)	64	2	NT	12—24	200	NT	480	1	NT	84	NT	NT	19	NT	NT
Egg-detergent (EG-B)	46	22—44	270	230	5	NT	50—100	2	6	52	26	10	390	4	4

a) Experimental conditions are described in the text. The classification of carbohydrate-binding proteins was performed under the same conditions as in Table I. Numbers indicate the minimum quantity of lectin fraction (μg/50 μl) that causes hemagglutination. *b*) C, buffer C-eluted (non-adsorbed) fraction. NT, not tested.

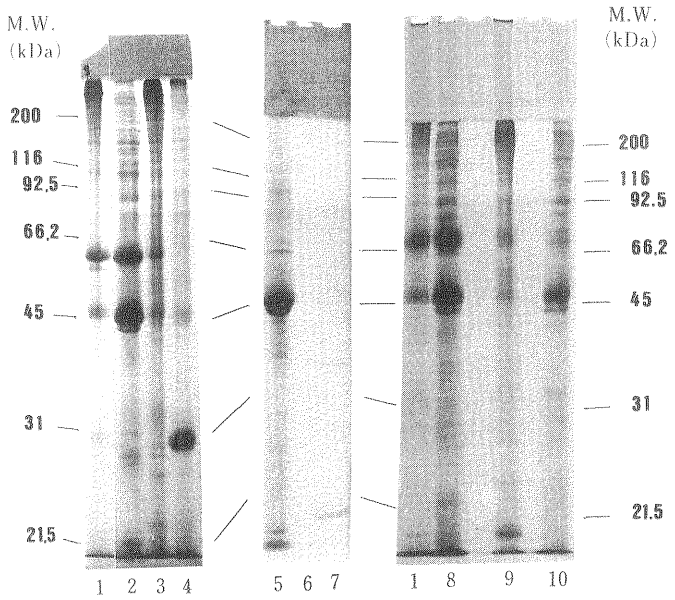

Fig. 2. SDS-PAGE of Galactose- and Lactose-Binding Proteins from "S1 Fraction"

Electrophoresis was performed as described in the legend to Fig. 1. Lane 1, S1 fraction; lane 2, GL-C; lane 3, GL-GD; lane 4, GL-LD; lane 5, GL-C'; lane 6, GL-GD'; lane 7, GL-LD'; lane 8, LG-C; lane 9, LG-GD; lane 10, LG-LD. The migration positions of molecular weight markers are indicated.

fraction from the rhamnose–Sepharose column, EG-AD fractions from lactose– and galactose–Sepharose columns, EG-BD fractions from lactose–, melibiose–, galactose–, mannose– and rhamnose–Sepharose columns, and EG-BE fractions from galactose–, mannose– and rhamnose–Sepharose columns. In addition, hemagglutinating activity was also observed in non-adsorbed SK-AC fraction from the mannose–Sepharose column and in non-adsorbed EG-AC fractions from melibiose– and rhamnose–Sepharose columns. On the other hand, neither SK-BD nor EG-BD fraction from the lactose–Sepharose column agglutinated trypsinized rabbit erythrocytes (data not shown).

Successive Linked Affinity Chromatography of "S1 Fraction" and "E1 Fraction" The S1 fraction was fractionated by affinity chromatography on two successive sugar–Sepharose linked columns, a galactose–Sepharose column connected to a lactose–Sepharose column (GL column) and a reverse type (LG column). When the GL column system was used, the hemagglutination activity was observed in

TABLE III. Hemagglutinating Activity toward Human A Erythrocytes and Molecular Weight of Galactose- and Lactose-Binding Proteins from "S1 Fraction"

Fraction	Lectin activity (minimum quantity of lectin fraction, μg protein, that causes agglutination)	Molecular weight of major component[a] (see Fig. 2)
S1 fraction	26	>200, 70, 49
GL-(buffer C) (GL-C)	90	49, 70, 45
GL-galactose (buffer D) (GL-GD)	10	>200, 70, 49
GL-galactose (buffer E) (GL-GE)	ND	—
GL-lactose (buffer D) (GL-LD)	6	30
GL-lactose (buffer E) (GL-LE)	52	—
GL-(buffer C') (GL-C')	17	49, 45, 19
GL-galactose (buffer D') (GL-GD')	300	37
GL-galactose (buffer E') (GL-GE')	ND	—
GL-lactose (buffer D') (GL-LD')	150	37, 21
GL-lactose (buffer E') (GL-LE')	ND	—
LG-(buffer C) (LG-C)	90	49, 72
LG-lactose (buffer D) (LG-LD)	14	49, 45, 72
LG-lactose (buffer E) (LG-LE)	ND	—
LG-galactose (buffer D) (LG-GD)	13	>200, 72, 19
LG-galactose (buffer E) (LG-GE)	ND	—

a) The molecular weight is given in kDa. ND, not determined.

TABLE IV. Agglutinating Activity toward MM46 Tumor Cells of Galactose- and Lactose-Binding Proteins from "E1 Fraction" a)

Fraction	Lectin activity (minimum quantity of lectin fraction, μg protein, that causes agglutination)
E1 fraction	46
GL-(buffer C) (GL-C)	140
GL-galactose (buffer D) (GL-GD)	20
GL-lactose (buffer D) (GL-LD)	14

a) MM46 cells were maintained by i.p. inoculation of 0.1 ml of ascites into C3H/He mice. The cells were harvested and washed in physiological saline by repeated centrifugation, and the cell suspension (10^7 cells/ml) in physiological saline was used for lectin agglutination. The agglutinating activity was determined as described in the text.

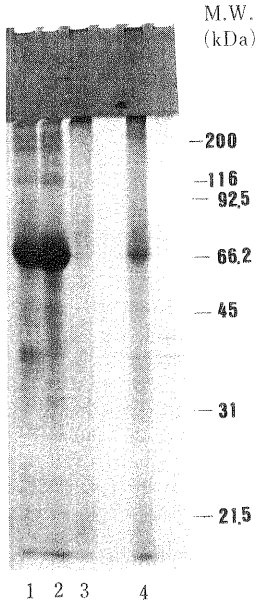

Fig. 3. SDS-PAGE of Galactose- and Lactose-Binding Proteins from "E1 Fraction"

Electrophoresis was performed as described in the legend to Fig. 1. Lane 1, E1 fraction; lane 2, GL-C; lane 3, GL-GD; lane 4, GL-LD. The migration positions of molecular weight markers are indicated.

buffer D-eluted fractions of both the galactose–Sepharose and lactose–Sepharose columns (GL-GD fraction and GL-LD fraction) (Table III). According to SDS-PAGE analysis, a band of 30 kDa accounted for over 80% of the total protein in the GL-LD fraction (see Fig. 2). Although the GL-GD fraction contained many bands, such as above 200, 70 and 49 kDa, no 30 kDa band was present. The hemagglutinating activities of the LG-LD and LG-GD fractions obtained from the LG column system were similar to those of the fractions from the GL column system, while the major components migrated as bands of M.W. = 49 and 45 kDa, and as bands of M.W. = 72 and 19 kDa, respectively. As mentioned above, the hemagglutinating activity was concentrated in the buffer D-eluted fractions on addition of DTT and Triton X-100 to the buffers, although the fractions obtained in the absence of DTT and Triton X-100 were inactive.

The E1 fraction was also fractionated by affinity chromatography on the GL column system in the same manner as described above. The agglutinating activity against MM46 cells was found in buffer D-eluted fractions from both galactose–Sepharose and lactose–Sepharose columns (GL-GD fraction and GL-LD fraction). The major components of GL-GD and GL-LD fractions migrated as bands of M.W. = 76 and 20 kDa, and as bands of M.W. = 47 and 38 kDa, respectively (Table IV, Fig. 3).

Discussion

SDS-PAGE analysis (see Fig. 1 and Table I) indicated that many sugar-binding proteins were contained in *X. laevis* skin and eggs. When various affinity adsorbents having a galactosyl residue (galactose–Sepharose, lactose–Sepharose and melibiose–Sepharose) were employed for the separation of galactose-binding proteins, the adsorbed proteins detected by SDS-PAGE differed in each case. Since the purification of lectins by single sugar–Sepharose column chromatography has not proved consistently successful, it was thought advisable to investigate the use of affinity chromatography based upon combinations of a few affinity adsorbents for the isolation of lectins on the basis of differences of sugar specificity.

In previous work, we described the reactivities of three lectins extracted from *X. laevis* skin toward various cells.[10,21] Among them, the lectin derived from S1 fraction agglutinated human erythrocytes without showing any specificity for A, B and O blood groups, but had no effect on tumor cells. Hemagglutination of A erythrocytes by the S1 fraction was inhibited by lactose and lactulose but not by D-fucose, L-arabinose and D-galactose,[10] but no hemagglutinating activity bound to an affinity column of lactamyl-Sepharose[22] or acid-treated Sepharose[23] equilibrated with PBS (data not shown). Since the sugar-binding proteins were adsorbed on the affinity column in the presence of DTT and Triton X-100 (Figs. 1 and 2, Tables I and III), the requirement of a reducing agent for maintaining binding activity to the adsorbent of sugar-binding proteins reported here is similar to those pointed out in the case of electrolectin,[24] chick embryo thigh muscle β-D-galactoside-specific lectin[25] and rat lung β-galactoside-binding lectin.[26] The GL column system was used for purification of hemagglutinin from the S1 fraction because several salt-extractable Ca^{2+}-dependent galactose-binding proteins from SK-A were trapped on galactose–Sepharose and a similar lactose-binding lectin of 30 kDa was concentrated in buffer D-eluted (SK-AD) fraction from the lactose–Sepharose column. The minimum quantity of the lectin of 30 kDa was 200 μg/well for agglutination of rabbit erythrocytes but 30 μg/well for that of human A erythrocytes (data not shown).

Bols *et al.* reported that injection of epinephrine induced marked secretion of 16 kDa lectin from *X. laevis* skin and that the 16 kDa lectin was purified by affinity chromatography on lactose–agarose by elution in the presence of lactose.[27] They noted a very faint anti-16 kDa lectin antibody-binding band with M.W. = 32 kDa, and suggested that this 32 kDa band may be an undissociated dimeric form of 16 kDa lectin. From the result presented here that a Ca^{2+}-dependent lactose-binding lectin of 30 kDa was separated from the S1 fraction by the GL column system in the presence of DTT and the report that a soluble 47

kDa hemagglutinin has been isolated from the S1 fraction by diethylaminoethyl(DEAE)-cellulose chromatography, PAGE and high-performance liquid chromatography on DEAE 5PW (Toyo Soda CCPM system) under DTT-free conditions,[28] it seemed reasonable to assume that the 30 or 47 kDa lectin molecule might be converted into the 16 kDa lectin molecule with a change of antigenicity. The conversion of 30 or 47 kDa lectin into 16 kDa lectin may be accomplished by digestion with proteolytic enzyme(s) which may be activated during the process of purification under reducing conditions, and not by the cleavage of disulfide linkages. Moreover, other molecule(s) may have a toxic effect when massive secretion from granular gland cells due to rupture of the plasma membrane is caused by the injection of epinephrine.

We also reported that an egg lectin from E1 fraction strongly agglutinated various tumor cells as well as sialidase-treated human erythrocytes.[11] Agglutination of S-180 cells by the lectin was inhibited by D-fucose, L-arabinose, D-galactose and lactose, even though this lectin had no affinity for lactamyl-Sepharose in PBS. Salt-extractable Ca^{2+}-dependent sugar-binding proteins, such as lactose-binding protein of 43 kDa, galactose-binding proteins of 47 and 39 kDa and melibiose-binding proteins of 45 and 38 kDa, required the presence of a reducing agent for the retention of binding affinity to the adsorbent under the conditions described above (Fig. 1 and Table I).

Although Roberson and Barondes reported that the melibiose-binding embryo or oocyte lectin has two subunits with apparent molecular weights of 45 and 43 kDa, and agglutinates trypsin-treated, glutaraldehyde-fixed rabbit erythrocytes,[29] we found that salt-extractable Ca^{2+}-dependent melibiose-binding proteins of 45 and 38 kDa, which may correspond to the two subunits of their embryo lectin, did not agglutinate either intact or trypsin-treated rabbit erythrocytes. If hemagglutination activity of melibiose-binding lectin was abolished by the DTT treatment, it is possible that the intact (undissociated) lectin molecule is required for the appearance of such activity.

Studies by Nishihara *et al.*[30] and Chamow and Hedrick[31] indicated that cortical granule-derived lectin from *X. laevis* eggs can be purified by affinity chromatography with its natural ligand, the egg jelly coat, and that this lectin contained 10—12 monomers, each of which had considerable charge and size heterogeneity (an average molecular weight of 45 kDa by SDS-PAGE) due to the presence of glycosylated side chains. Based on these findings, it is possible that the differences in the molecular weights of Ca^{2+}-dependent sugar-binding proteins, such as lactose-binding protein of 43 kDa, galactose-binding protein of 47 kDa and melibiose-binding protein of 45 kDa, are dependent on the heterogeneity of carbohydrate moieties of the subunit molecule.

Drickamer reported that animal lectins can be roughly divided into two distinct classes of carbohydrate-recognition domains, C-type (Ca^{2+}-dependent) and S-type (thiol-dependent) lectins.[32] Since the salt-extractable Ca^{2+}-dependent lactose-binding skin lectin of 30 kDa and the similar egg lectin of 43 kDa require the presence of DTT and calcium ion for binding to the lactose–Sepharose column, it seems reasonable to conclude that these lectins may have both C-type and S-type carbohydrate-recognition domains.

The findings presented here indicate that the presence of DTT may or may not be required for adsorption of particular sugar-binding proteins on sugar–Sepharose columns or for hemagglutinating activity of these proteins, and suggest that, if a lectin is an oligomeric molecule, one or more subunits which have no sugar-binding capacity may escape from the affinity column in the presence of DTT. We consider that further work should be done to purify such a lectin molecule possessing hemagglutinating activity and to dissociate it into the sugar-binding subunit and the other subunit so that the function of each subunit can be separately examined.

References and Notes

1) The following abbreviations are used: DVS, divinyl sulfone; DTT, dithiothreitol; PMSF, phenylmethanesulfonyl fluoride; EDTA ethylenediaminetetraacetic acid; buffer A, 20 mM Tris–HCl, pH 7.8, containing 1 mM DTT, 0.1 mM PMSF and 0.2 M NaCl; buffer B, 20 mM Tris–HCl, pH 7.8, containing 1 mM DTT, 0.1 mM PMSF, 0.4 M KCl and 2% Triton X-100; buffer C, 20 mM Tris–HCl, pH 7.8, containing 1 mM DTT, 1.25 M NaCl, 0.05% Triton X-100 and 25 mM $CaCl_2$; buffer D, buffer C containing 4 mM EDTA instead of 25 mM $CaCl_2$; buffer E, buffer C plus 0.2 M sugar; buffer A′, buffer A minus DTT; buffer C′, buffer C minus DTT and Triton X-100; buffer D′, buffer C′ containing 4 mM EDTA instead of 25 mM $CaCl_2$; buffer E′, buffer C′ plus 0.2 M sugar; GL column, galactose–Sepharose connected to lactose–Sepharose column; LG column, lactose–Sepharose connected to galactose–Sepharose column; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; PBS, phosphate-buffered saline; SK-A; buffer A (salt)-extractable fraction of skin; SK-B, buffer B (detergent)-extractable fraction of skin; EG-A, buffer A (salt)-extractable fraction of eggs; EG-B, buffer B (detergent)-extractable fraction of eggs; SK-AD, buffer D-eluted fraction of SK-A; SK-AE, buffer E-eluted fraction of SK-A; SK-BD, buffer D-eluted fraction of SK-B; SK-BE, buffer E-eluted fraction of SK-B, EG-AD, buffer D-eluted fraction of EG-A; EG-AE, buffer E-eluted fraction of EG-A; EG-BD, buffer D-eluted fraction of EG-B; EG-BE, buffer E-eluted fraction of EG-B; SK-AC, buffer C-eluted (non-adsorbed) fraction of SK-A; EG-AC, buffer C-eluted fraction of EG-A; GL-GD, buffer D-eluted fraction of galactose–Sepharose on GL column system; GL-GE, buffer E-eluted fraction of galactose–Sepharose on GL column system; GL-LD, buffer D-eluted fraction of lactose-Sepharose on GL column system; GL-LE, buffer E-eluted fraction of lactose–Sepharose on GL column system; GL-GD′, buffer D′-eluted fraction of galactose–Sepharose on GL column system; GL-GE′, buffer E′-eluted fraction of galactose–Sepharose on GL column system; GL-LD′, buffer D′-eluted fraction of lactose–Sepharose on GL column system; GL-LE′, buffer E′-eluted fraction of lactose–Sepharose on GL column system; LG-LD, buffer D-eluted fraction of lactose–Sepharose on LG column system; LG-LE, buffer E-eluted fraction of lactose–Sepharose on LG column system; LG-GD, buffer D-eluted fraction of galactose–Sepharose on LG column system; LG-GE, buffer E-eluted fraction of galactose–Sepharose on LG column system; GL-C, buffer C-eluted (non-adsorbed) fraction of GL column; GL-C′, buffer C′-eluted fraction of GL column; LG-C, buffer C-eluted fraction of LG column.
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