Carbohydrate Research, 108 (1982) 148–152
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Purification of lectin from some shellfish and ascidiacea

MASAHIRO TATSUMI, NORIKO HOSOKAWA, YUJI ARAI, AND TATSURO ITOH
Institute of Applied Biochemistry, The University of Tsukuba, Niihari-gun, Ibaraki 305 (Japan)
(Received November 2nd, 1981; accepted for publication, February 12th, 1982)

Although many lectins are available for studies of cell membranes and glycosylated materials, and they are widely used as ligands in affinity chromatography, their structures have not yet been fully elucidated. Johnson[1] described a lectin from butter clams (*Saxidomus giganteus*) which specifically agglutinated human red-blood cells of type A. Tripp[2] reported the presence of a hemagglutinin in the oyster *Crassostrea virginica*, and its chemical and physical properties have been investigated[3,4]. About half of a hundred species of shellfishes were found to have hemagglutination activity[5]. However, few shellfish lectins have been purified to homogeneity. Ascidiacea lectins from *Styela plicata* and *Halocynthia hilgendorf* have been reported by Fuke and Sugai[6].

We have determined the hemagglutination activities to human erythrocytes [types A, B, and H (O)] of several shellfishes and some ascidiaceas (Table I) and purified the lectins. The hemagglutination activities were found in three shellfishes and one ascidiacea. All of the lectins isolated specifically agglutinated erythrocytes of type A and the strongest activity was found in *Saxidomus purpuratus*. Lectins of similar nature were isolated from *Saxidomus giganteus* (*cf.* ref. 1) in *Saxidomus purpuratus*, suggesting that the *Saxidomus* group may contain lectin that specifically agglutinated type A erythrocytes, regardless of its habitat. The ascidiacea lectin *Cyntia ritteri* also showed hemagglutinin activity for red-blood cells of type A. Although Fuke and Sugai[6] have reported that *Styela plicata* has agglutination activity for rabbit erythrocytes, it did not agglutinate human erythrocytes of type A, B, and H.

The animal bodies were homogenised with Dulbecco's phosphate-buffered saline (2 vol.) and centrifuged (10,000*g*, 30 min), and the extracts were subjected to affinity chromatography on 2-acetamido-2-deoxy-D-galactose–Sepharose. The lectins were eluted with 0.1M GalNAc, and Fig. 1 shows the electrophoresis pattern of the purified lectins on polyacrylamide gel. Each lectin gave several protein bands and that from *Spisula sachalinensis* gave many protein bands.

Since *Saxidomus purpuratus* has a high content of lectin (SPA, *Saxidomus*

Abbreviation: SPA, *Saxidomus purpuratus* lectin purified by affinity chromatography.

TABLE I

HEMAGGLUTINATION ACTIVITIES OF SHELLFISHES AND ASCIDIACEAS FOR HUMAN ERYTHROCYTES OF TYPES A, B, AND H

	Erythrocyte type		
	A	*B*	*H*
Anadara broughtonii	—	—	—
Atrina pectinata	—	—	—
Babyronia japonica	—	—	—
Corbicula japonica	—	—	—
Hyriopsis schlegeli	—	—	—
Mactra chnensis	—	—	—
Meretrix lusoria	+	—	—
Neptunea lyrata	—	—	—
Notohaliotis discus	—	—	—
Patinopecten yessoensis	—	—	—
Saxidomus purpuratus	+	—	—
Spisula sachalinensis	—	—	—
Sulculus supertexta	—	—	—
Tapes japonica	—	—	—
Tresus keenae	—	—	—
Turbo cornutus	—	—	—
Botryllus primigenus	—	—	—
Cynthia ritteri	+	—	—
Cynthia roretzi	—	—	—
Styela plicata	—	—	—

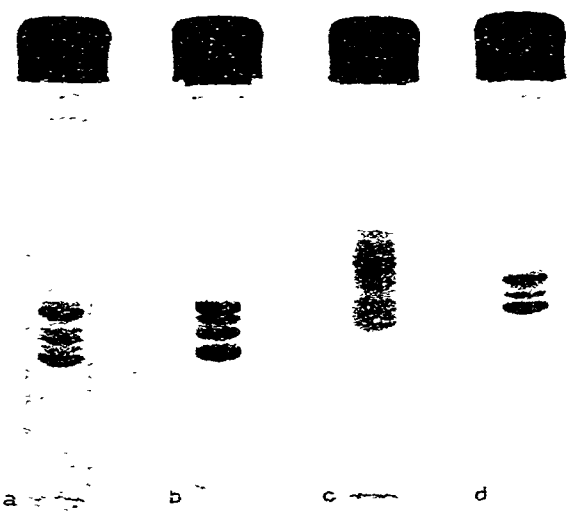

Fig. 1. Polyacrylamide gel electrophoresis of lectins that were purified by affinity chromatography: (*a*) *Saxidomus purpuratus*, (*b*) *Meretrix lusoria*, (*c*) *Spisula saxhalinensis*, and (*d*) *Cynthia ritteri*.

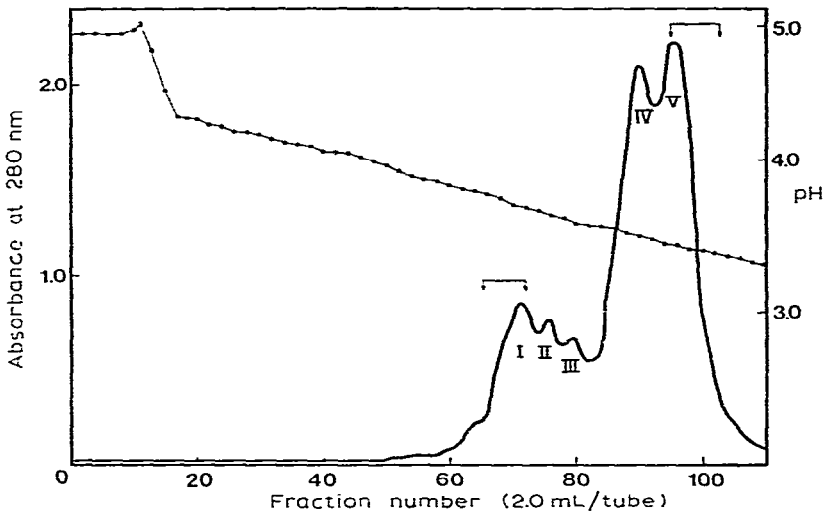

Fig. 2. Chromatography of SPA on PBE 74 (Polybuffer exchanger). Indicated groups of fractions were combined separately: ———, absorbance at 280 nm; —●—●—●—, pH.

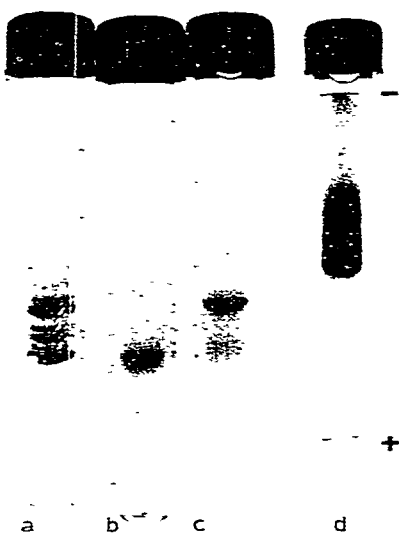

Fig. 3. Polyacrylamide gel electrophoresis of *Saxidomus purpuratus* lectin: (*a*) lectin purified by affinity chromatography, (*b*) and (*c*) the purified lectins from fractions I and V of chromatography on PBE 74, (*d*) isoelectric focusing of SPA on a Pharmalite gradient [pH 5.0 (top) to pH 2.5 (bottom)].

purpuratus agglutinin), it was subjected to chromatofocusing and eluted with Polybuffer 74 (Fig. 2). Hemagglutination activity was found in each of the five protein peaks. To examine the homogeneity of the active fraction, each alternate fraction was subjected to analytical electrophoresis on polyacrylamide gel. The first half of peak I and the last half of peak V gave a single protein band. These two fractions were dialysed against water. The hemagglutination activity of peak-I fraction was purified

~74 fold with a yield of 6.1%, and the hemagglutination activity of peak V fraction was purified ~150 fold with a yield of 18%. Fig. 3 shows the electrophoresis pattern of the purified lectins on polyacrylamide gel.

Table II shows the inhibition by sugars of hemagglutination induced by SPA. The hemagglutination was inhibited by a low concentration of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, but not significantly by low concentrations of D-glucose, D-galactose, D-mannose, L-fucose, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, or 2-amino-2-deoxy-D-mannose. Although GalNAc is one of the best inhibitors of hemagglutination induced by A-specific lectins, the best inhibitor of SPA in this study was found to be methyl 2-acetamido-2-deoxy-α-D-glucopyranoside. This result suggests that SPA and *Bandeiraea simplicifolia* II lectin[7] had very similar sugar affinities.

The molecular weight of SPA was estimated to be 40,000 by gel filtration on Sephadex G-150. Though SPA consisted of at least seven proteins (Fig. 3a), gel filtration gave a single, symmetrical protein peak. Isoelectric focusing of SPA in polyacrylamide gel (pH gradient, 2.5–5) gave four major protein bands in the pH range 4.1–4.5 (Fig. 3d). The lectin showed microheterogeneity with respect to its electrical properties. Microheterogeneity has been reported for hemagglutinins of several origins, *e.g.*, *Vicia graminea*[8], *Crassostrea virginica*[2], wheat-germ agglutinin[9], and *Helix pomatia*[10]. Different contents of sialic acid may be the cause of microheterogeneity in various animal glycoproteins, and microheterogeneity attributable to the carbohydrate groups in glycoprotein has been reported[12]. However, no sialic acid was detected in SPA by the methods of Aminoff[13] with *N*-acetylneuraminic acid as a standard, so that the microheterogeneity must have another origin.

EXPERIMENTAL

Affinity chromatography. — GalNAc was attached to epoxy-activated Sepharose

TABLE II

INHIBITION OF PURIFIED *Saxidomus purpuratus* LECTIN FOR TYPE A ERYTHROCYTES WITH SUGARS

	Minimum concentration (mM) completely inhibiting 4 hemagglutination doses	
	Fraction I	*Fraction V*
2-Acetamido-2-deoxy-D-glucose	0.8	1.0
2-Acetamido-2-deoxy-D-galactose	150	180
2-Acetamido-2-deoxy-D-mannose	220	220
2-Benzamido-2-deoxy-D-glucose	>300	>300
Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside	0.15	0.25
Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside	1.0	2.0
Di-*N*-acetylchitobiose	>50	>50

4B by the method of Matsumoto *et al.*[14].

Polyacrylamide gel electrophoresis. — Disc gel electrophoresis was carried out by the method of Williams and Reisfeld[15] by using a 7.5% polyacrylamide gel at pH 8.0 and staining for protein with Coomassie Brilliant Blue G.

Chromatofocusing. — Chromatofocusing gel PBE 94 (Pharmacia) was equilibrated with 25mM piperazine buffer (pH 5) and packed in a column (1.0 × 30 cm). The sample, previously dialysed against the same buffer, was applied on the column and eluted with 220 mL of Polybuffer 74 (diluted 10 times, pH 3.5).

Isoelectric focusing. — Analytical isoelectric focusing was carried out[16] by using 5% polyacrylamide gels containing 2% of Pharmalite (pH 2.5–5, Pharmacia).

Molecular weight determinations. — Analytical gel filtration was performed by the method of Andrews[17] by using a column (1.8 × 98 cm) of Sephadex G-150 equilibrated with 10mM Tris-HCl buffer (pH 7.4) containing 0.15M NaCl. The column was eluted with the same buffer at 12 mL/h at 25°. The molecular weight of the lectin was estimated by comparison of its distribution coefficient (K_d) with those of bovine serum albumin (mol. wt. 68,000), ovalbumin (45,000), chymotrypsinogen A (25,000), and cytochrome C (12,500).

Hemagglutinin and hemagglutinin-inhibition assays. — Hemagglutinin was titrated[18] by serial dilution. Hemagglutinin-inhibition assays were carried out as described by Matsumoto and Osawa[19]. Inhibiting activity was expressed as the lowest concentration of a substance in the incubation mixture which completely inhibited agglutination.

REFERENCES

1 H. M. Johnson, *Science*, 146 (1964) 548–549.
2 M. R. Tripp, *J. Invertebr. Pathol.*, 8 (1966) 478–484.
3 J. E. McDade and M. R. Tripp, *J. Invertebr. Pathol.*, 9 (1967) 523–530.
4 R. T. Acton, J. C. Benett, E. E. Evans, and R. E. Schrohenloher, *J. Biol. Chem.*, 244 (1969) 4128–4135.
5 R. T. Pemberton, *Ann. N.Y. Acad. Sci.*, 234 (1974) 95–121.
6 M. T. Fuke and T. Sugai, *Biol. Bull.*, 143 (1972) 140–149.
7 P. N. S. Iyer, K. D. Wiklinson, and I. J. Goldstein, *Arch. Biochem. Biophys.*, 177 (1976) 330–333.
8 M. J. Prigent and R. Bourrillon, *Biochem. J.*, 189 (1980) 185–188.
9 A. K. Allen, A. Neuberger, and N. Sharon, *Biochem. J.*, 131 (1973) 155–162.
10 S. Hammarstrom and E. A. Kabat, *Biochemistry*, 8 (1969) 2696–2705.
11 E. F. Zimmerman, D. Bowen, J. R. Wilson, and M. M. Medappally, *Biochemistry*, 15 (1976) 5534–5543.
12 J. F. McKelvy and Y. C. Lee, *Arch. Biochem. Biophys.*, 132 (1969) 99–100.
13 D. Aminoff, *Biochem. J.*, 81 (1961) 384–392.
14 I. Matsumoto, Y. Mizuno, and N. Seno, *J. Biochem. (Tokyo)*, 85 (1979) 1091–1098.
15 D. E. Williams and R. A. Reisfeld, *Ann. N.Y. Acad. Sci.*, 121 (1964) 378–381.
16 P. Doerr and A. Chrambach, *Anal. Biochem.*, 42 (1971) 96–107.
17 P. Andrews, *Biochem. J.*, 96 (1965) 595–606.
18 E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry*, 2nd edn., Charles C. Thomas, Springfield, IL, 1961.
19 I. Matsumoto and T. Osawa, *Biochim. Biophys. Acta*, 194 (1969) 180–189.