KOREAN J. FOOD SCI. TECHNOL.
Vol. 21, No. 5, pp. 606~612 (1989)

Purification and Biophysical Characterization of New Lectin from Baby Clam, *Tapes japonica*

Hee-Sook Kim

Department of Food Science and Technology, Kyoungsung University, Pusan

Abstract

A New lectin from baby clam, *Tapes japonica*, was isolated and purified through the following procedures; acetone powder, 0.15M NaCl extraction, ammonium sulfate fractionation, N-acetyl-D-galactosamine-agarose affinity column, and ion exchange Mono Q of FPLC. This lectin nonspecifically agglutinated human erythrocytes but didn't agglutinate mouse and rabbit erythrocytes. And the lectin neither stimulated human lymphocytes nor agglutinated Sarcoma 180 cells. On polyacrylamide gel electrophoresis, the lectin migrated as a major single band indicating homogeneous. A molecular weight was estimated to be about 131,000 daltons by Biogel P-300 and 125,000 daltons by SDS-PAGE without β-mercaptoethanol. This lectin is supposed to be a tetramer composed of heterogeneous subunits, about 30,000 and 33,000 daltons. Baby clam lectin was inhibited by EDTA and recovered agglutinating activity by Ca^{++} and Mn^{++}. This lectin is revealed as glycoprotein that contained about 4.2% neutral sugar.

Key words: baby clam lectin

Introduction

Lectins, sugar-specific binding proteins or glycoproteins, have proven to be quite useful in biological and medical researches because of their hemagglutinating, mitogenic, and toxic properties[1-5].

Ricin, a highly toxic protein present in castor beans, inhibits protein synthesis in a cell-free system even though it is present in extremely small concentration[6]. Several plants lectins investigated to cytolysis or agglutinate tumor cells[7,8]. Recently, many lectins have purified and characterized from invertebrate, *Helix pomatia*[9], *Limulus polyphemus*[10], *Homarus americanus*[11], *Neptunea intersculpta*[12] and *Mytilus edulis*[13]. These invertebrate lectins have found in the albumin glands, muscles or hemolymphs. Tapes japonica contained hemagglutinins showing nonspecificity for human erythrocytes. One lectin of *Tapes japonica* has been purified and characterized[14]. In this paper, I wish to report on the purification and characterization of isolectin from *Tapes japonica*.

Corresponding author: Hee-Sook Kim, Department of Food Science & Technology, Kyoungsung University, Daeyoun-dong, Nam-gu, Pusan 608-736

Materials and Methods

Materials

Fresh baby clam, *Tapes japonica*, was purchased from Jagalchi fish market in Pusan. Fresh blood was drawn out from healthy people and animals.

Lectin purification

Acetone powder of *Tapes japonica* by homogenizing with acetone and ice and lyophilizing. Crude lectin was extracted by 0.15M NaCl from acetone powder and precipitated by ammonium sulfate fractionation (0-70% saturation). Ammonium sulfate fraction was dialyzed against 25 mM Tris-HCl (pH 7.2) and applied to a N-acetyl-D-galactosamine-agarose column (1.3×20 cm) which was equilibrated with 25 mM Tris-HCl (pH 7.2). The column was washed with Tris-HCl buffer until the absorbancy at 280 nm of the eluate fell below 0.01. The absorbed lectin, eluted with

0.5M NaCl containing 2.5 mM GalNAc and 25 mM Tris-HCl (pH 7.2) buffer, was pooled and dialyzed against 25 mM phosphate buffer (pH 6.5). The dialyzate was applied to anion exchange column Mono Q of Fast Protein Liquid Chromatography (FPLC). The active fraction was dialyzed throughly against 25 mM Tris-HCl buffer (pH 7.2) and distilled water and concentrated.

Immunochemical study

Rabbit anti-lectin serum was prepared by immunizing white rabbit with purified lectin on affinity column[15]. Immunodiffusion was performed on 1% agar plate containing 0.15M NaCl and 0.02% NaN_3 by Ouchterlony's method[15]. The gel washed with 0.15M NaCl and stained Coomassie Brilliant Blue R-250.

Effects of metal ions and EDTA on hemagglutinating activity

Baby clam lectin was inhibited by chelate compound, EDTA(14), so we tested whether some metal ions recovered hemagglutinating activity or not. One-half lectin was dialyzed twice against 0.15M NaCl, 25 mM Tris-HCl, and 10 mM EDTA. After dialyzed lectin was serially diluted with saline, each metal ion (10 mM) was added to each well and mixed thoroughly. And then, a 3% suspension of erythrocytes was added and incubated for 1 hr.

Disc polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was performed according to the method of Davis[16] using Tris-Glycine buffer (pH 8.3) and 7.5% polyacrylamide gels to evaluate the purity of the lectin. Proteins were detected with 0.1% Coomassie Brilliant Blue.

SDS-Polyacrylamide gel electrophoresis

The molecular weight of the subunits was estimated by SDS-PAGE according to Weber and Osborn[17]. A gel was stained with 0.1% Coomassie Brilliant Blue for protein detection and another gel was stained by periodic acid-Schiff stain method according to Grossmann[18] for glycoprotein.

Gel filtrtion for molecular weight

Gel filration was carried out by the method of Andrew by using a Biogel p-300 column(1.6× 120cm) equilibrated with 50mM Tris-HCl buffer (pH 7.2) containing 0.5M NaCl. The molecular weight of the lectin was assessed from the calibration curve which was prepared by standard proteins.

Isoelectrofocusing

For determination of the pI value of the lectin, continous gel isoelectric focusing(Pharmacia Fine Chem.) was performed in T 5%, C 3% polyacrylamide gel containing 6.25% Pharmalyte(pH 3 to 10). The isoelectric point of the lectin was determined on a graph of the relation between the mobility of maker proteins and their isoelectric points.

Determination of protein

Protein concentration was defined by the method of Lowry *et al.*[19] with bovine serum albumin as a standard.

Determination of carbohydrate

Total carbohydrate content was determined by the phenol-sulfuric acid procedure[20] using arabinose as a standard.

Ultraviolet absorption

The ultraviolet absorption spectra of the lectin was determined in an aqueous solution with Shimadzu Model UV/Vis-240 Spectrophotometer.

Results and Discussion

Purification of lectin

Baby clam lectin was purified by acetone powder, 0.15M NaCl extraction, ammonium sulfate fractionation (0-70% saturation), affinity chro-

Table 1. Purification of baby clam lectin

Procedure	Total protein (mg)	Total units ($\times 10^3$)	Specific activity (units/mg)	Purification (fold)	Recovery (%)
20,000×g homogenate	4563	3840	841.55	1	100
0.15M extract	2012	3200	1590.46	1.89	83.3
$(NH_4)_2SO_4$ fraction	1123	2304	2051.65	2.44	60.0
Affinity column	6.8	704	103529.4	123.02	18.3
Mono Q column of FPLC	4.2	569.6	135619.05	161.15	14.8

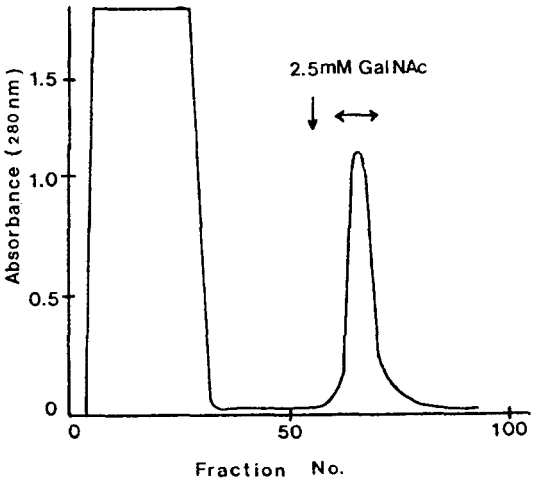

Fig. 1. Elution pattern of the N-acetyl-D-galactosamine-agarose affinity chromatography.
The hemagglutinating activity of lectin fraction (↔) was measured after dialysis. Column, 1.3×20 cm; Flow rate, 10 ml/h.

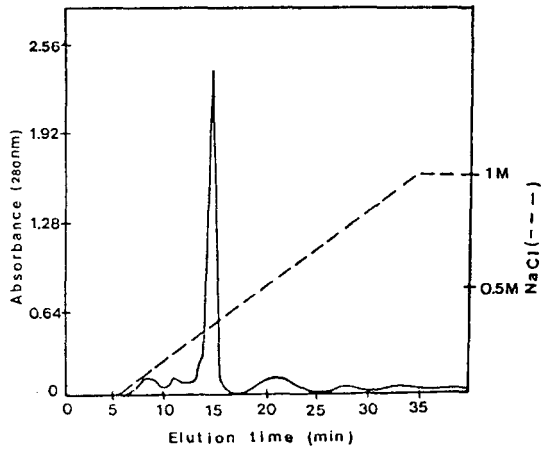

Fig. 2. Elution pattern of the Mono Q column chromatography of FPLC(Pharmacia Fine Chemicals).
NaCl linear gradient range, 0-1.0 M; Scale range, 2.56; OD of sample, 1.6; Sample injection, 1.3 ml; Flow rate, 1 ml/min.

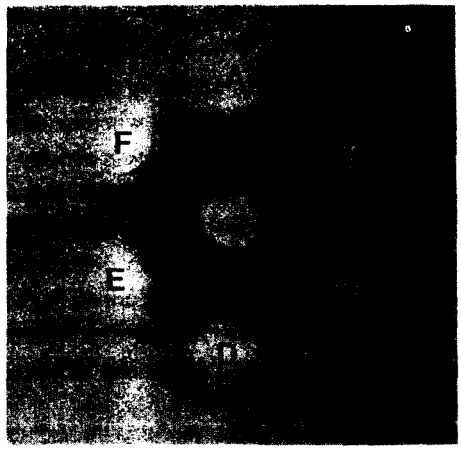

Fig. 3. Immunodiffusion of rabbit anti-lectin serum (central well) with the partial purified lectin.
A,B; $(NH_4)_2SO_4$ fraction (A, 25μl; B, 50μl), D; Affinity column fraction, E; Mono Q column fraction.

matography and Mono-Q column of FPLC. The results of these purification steps are summarized in Table 1. Fig. 1 shows the elution pattern of the affinity chromatography of N-acetyl-D-galactosamine-agarose column. The bound materials were eluted with 2.5mM GalNAc and the activity was determined after dialyzed. On Mono-Q column of FPLC, the lectin was eluted by a linear gradient of NaCl from 0.3 to 0.35M (Fig. 2). From these purification procedures, specific activity of baby clam lectin was increased from 841.55 to 135619.05 units/mg. In the polyacrylamide gel electrophoresis, lectin showed one major band. The result of Ouchterlony double immunodiffusion test is shown in Fig. 3. The one sharp precipitin line between antiserum and FPLC fraction also suggested the lectin was probably purified homogeneously as the result from gel electrophoresis.

Table 2. Agglutinating and stimulating activity of baby clam lectin.

Cells	Relative activity[b](%)
Erythrocytes[a]	
Human type A	100
type B	100
type AB	25
Mouse	No agglutination
Rabbit	No agglutination
Human lymphocytes	No stimulation
Sarcoma acite cells	No agglutination

a) These erythrocytes were trypsinized at 37°C for 1 hr.
b) The hemagglutinating activity with human type A erythrocytes was taken as 100%.

Agglutinating activity

As shown in Table 2, baby clam lectin agglutinated human erythrocytes nonspecifically. But erythrocytes of mouse and rabbit was not agglutinated. This lectin didn't stimulate lymphocytes and not agglutinate Sarcoma 180 cells. It was reported that Con A from Jack bean and *Lentil* lectin stimulated the transformation of T lymphocyte[3] and PHA from red kidney bean and american lobster lectin stimulated B lymphocyte[4]. Ricin from castor bean agglutinated Sarcoma 180 cells and another tumor cells[5,6]. Treatment of purified lectin with EDTA inhibited agglutinating activity and the readdition of Ca^{++} to the functionally inactive molecule resulted to recover agglutinating activity (Table 3). The addition of Mn^{++} to the inactive lectin also showed to recover agglutinating activity slightly.

Table 3. Effects of the metal ions against erythrocyte-agglutinating function of the purified lectin.

Sample preparation	Titer[a]
Lectin alone	2^5
Lectin + 10 mM $CaCl_2$	2^5
Lectin + 10 mM EDTA	Negative
Lectin + 10 mM $CaCl_2$ after EDTA treatment	2^4
Lectin + 10 mM $MnCl_2$ after EDTA treatment	2^2
Lectin + 10 mM $CaCl_2$ + $MnCl_2$ after EDTA treatment	2^4

a) Baby clam lectin titers were performed against 3% trypsinized human A cells.

Fig. 4. SDS-PAGE in a 10% polyacrylamide rod gel of baby clam lectin.
Lane A, denaturation lectin without β-mercaptoethanol; Lane B, staining with Coomassie Blue of lectin subunits; Lane C, staining with Shiff base of lectin subunits

Molecular weight

On the SDS-PAGE, baby clam lectin showed two bands as shown in Fig. 4. The molecular weight of these two bands were estimated to about 30,000 and 33,000 daltons. When the lectin was heated without β-mercaptoethanol, the molecular weight was 125,000 daltons. On the staining with Coomassie Blue and periodic acid-Schiff base, the color densities of two bands were almost same (Fig. 4,5).

From Biogel P-300 column gel filtration, the molecular weight was calculated as about 131,000. On the basis of these results, it is supposed that the active form of baby clam lectin is tetrameric protein heterogeneously (Fig. 6).

Isoelectric point

From the results of analytical isoelectric focusing of the lectin on a Pharmalyte-polyacrylamide gel, its isoelectric point, pI, was determined to be

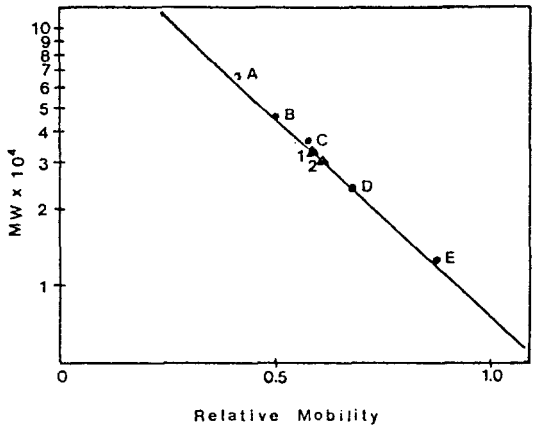

Fig. 5. Determination of molecular weight of lectin by SDS-PAGE.
A, bovine serum albumin (66,000); B, egg albumine (45,000); C, glyceraldehyde-3-phosphate dehydrogenase (36,000); D, trypsinogen (24,000); E, α-lactalbumin (14,200); 1,2, baby clam lectin subunits.

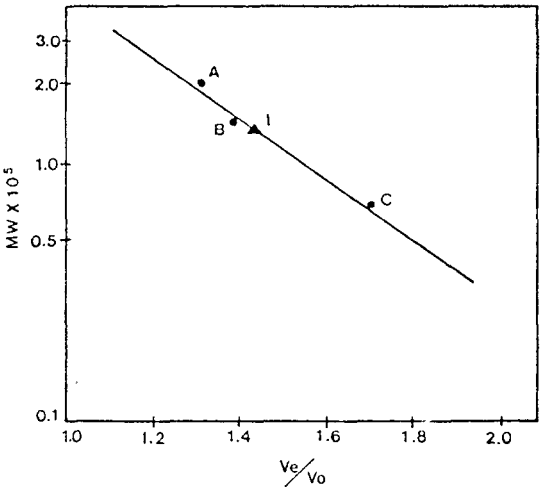

Fig. 6. Determination of molecular weight of lectin by gel filtration on Biogel P-300.
A, β-amylase (200,000); B, alcohol dehydrogenase (150,000); C, bovine serum albumin (66,000); 1, baby clam lectin.

about pH 6.25 (Fig. 7).

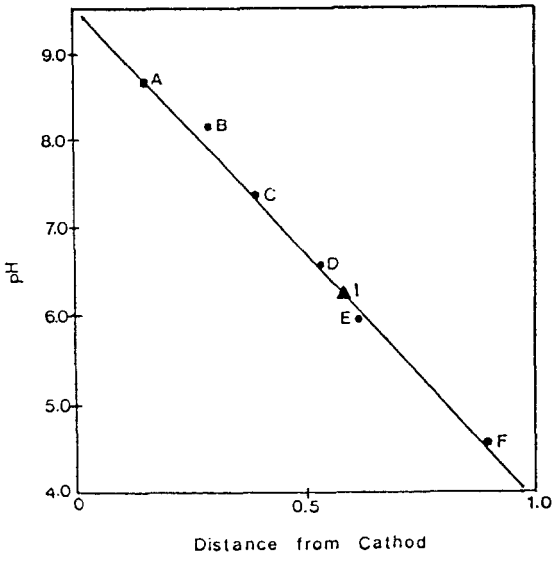

Fig. 7. Determination of isoelectric point of lectin by isoelectric focusing.
A, lentil lectin basic band (pI 8.65); B, lentil lectin acidic band (pI 8.15); C, horse myoglobin (pI 7.35); D, human carbonic anhydrase B (pI 6.55); E, bovine carbonic anhydrase B (pI 5.85); F, soybean trypsin inhibitor (pI 4.55); 1, baby clam lectin (pI 6.25)

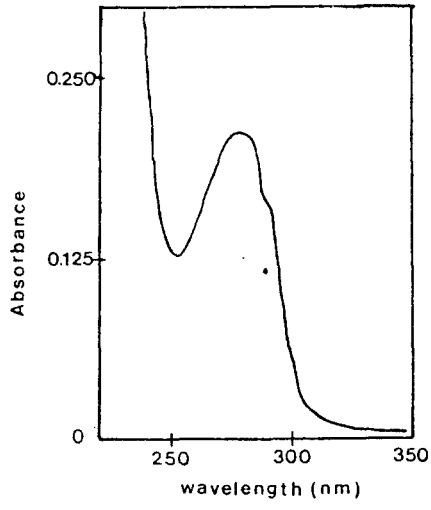

Fig. 8. Ultraviolet spectrum of baby clam lectin.

Carbohydrate content

Baby clam lectin was proved as glycoprotein according to periodic acid-Schiff staining of SDS-PAGE. This lectin contained about 4.2% as pentose, arabinose. When the spectrum profile was checked, the absorbance of 480 nm was stronger than that of 490 nm. So pentose was chosen as the carbohydrate standard.

Ultraviolet spectrum

The ultraviolet spectrum of baby clam lectin appeared in Fig. 8. The shoulder at 289 nm revealed that the lectin contained some tryptophane[21].

Acknowledgement

I wish to give sincere thanks to Professor Funatsu G. of Department of Agricultural Chemistry, Kyushu University, Japan.

References

1. Dixon, H.B.F.: Defining a lectin. *Nature*, **292**, 192 (1981)
2. Levi, G. and Teichburg, V.I.: Isolation and physicochemical characterization of electrolectin, a β-D-galactoside binding lectin from the electric organ of *Electrophorus electricus*. *J. Biol. Chem.*, **256**, 5735 (1981)
3. Jacobsson, H. and Blomgren, H.: Responses of mouse thymic cells to mitogens. *Cellular Immunol.*, **11**, 427 (1974)
4. Campbell, P.A., Hartman, A.L. and Abel, C.A.: Stimulation of B cells, but not T cells or thymocytes, by a sialic acid-specific lectin. *Immunology*, **45**, 155 (1982)
5. Funatsu, G., Miyauchi, S. and Funatsu, M.: Biochemical studies on Ricin: Effect of the two constituent polypeptide chains of Ricin D toward mouse Sarcoma acite tumor cells. *Agri. Biol. Chem.*, **3**, (3), 639 (1976)
6. Olsnes, S. and Pihl, A.: Different biological properties of the two constituent peptide chains of Ricin, a toxic protein inhibiting protein synthesis. *Biochemistry*, **12**(16), 3121 (1973)
7. Anaclerio, A., Waterfield, J.D. and Moller, G.: Induction of lymphocyte-mediated cytotoxicity against allogenic tumor cells by Con A *in vivo*. *J. Immunology*, **113**, 870 (1974)
8. Eckhardt, A.E. and Goldstein, I.J.: Occurence of α-D-galactosyl-containing glycoproteins on Ehrlich tumor cell membrane. *Biochemistry*, **22**, 5280 (1983).
9. Hammarström, S.: Snail (*Helix pomatia*) hemagglutinin *in Methods in Enzymol.*, Academic Press, New York, **28**, 368 (1974)
10. Finstad, C.L., Good, R.A. and Litman, G.W.: The erythrocyte agglutination from *Limulus polyphemus* homolymph: Molecular structure and Biological function. *Ann. N.Y. Acad. Sci.*, **234**, 170 (1974)
11. Hall, J.L. and Rowlands, D.T.: Heterogeneity of Lobster agglutinins I. *Biochemistry*, **13**, 821 (1974)
12. Suh-Chae, Y.A., Jeune-Chung, K.H. and Chung, S.R.: Purification and Biophysicochemical characterization of new lectin from marine shell, *Neptunea intersculpta. Ph.D. Thesis*, Yeungnam University, Korea (1987)
13. Park, J. and Kim, H.: Isolation and characterization of a lectin from the mussel, *Mytilus edulis*. *Korean Biochem. J.*, **20**(3), 208 (1987)
14. Kim, H.S.: Characterization of lectin isolated and purified from baby clam, *Tapes japonaca. Ph. D. Thesis*, Pusan National University, Korea (1987)
15. Dresser, D.W.: Immunization of experimental animals *in Handbook of Experimental Immunology* I(Weir D.M. ed.). Blackwell Scientific Publications, Oxford, Chapter 8.1 (1986)
16. Davis, B.J.: Disc electrophoresis II. *Ann. N.Y. Acad. Sci.*, **121**, 404 (1964)
17. Weber, K. and Osborn, M.: The reliability of molecular weight determination by dodesylsulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.*, **244**, 4406 (1969)
18. Grossman, H. and Neville, D.M.: Glycoprotein of cell surface. *J. Biol. Chem.*, **246**, 6339 (1971)
19. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.*, **193**, 265 (1951)
20. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F.: Colorimetric method for determination of sugar and related substances. *Anal. Chem.*, **28**, 350 (1956)
21. Edelhoch, H.: Spectroscopic determination of tryptophan and tyrosine in protein. *Biochemistry*, **6**(7), 1948 (1967)

(Received Mar. 29, 1989)

바지락(*Tapes japonica*)으로부터 분리정제된 새로운 렉틴의 생물물리학적 특성

김 희 숙
경성대학교 공과대학 식품공학과

한국산 바지락으로부터 새로운 렉틴을 아세톤파우더, 황산암모늄 침전, 친화력 크로마토그라피 및 FPLC의 이온교환 크로마토그라피법으로 분리 정제하였다. 이 렉틴은 사람의 적혈구를 비특이적으로 응집시켰으며, 생쥐와 토끼의 적혈구 및 생쥐의 복수 Sarcoma 180 세포를 응집시키지 않았고 사람의 말초혈관 임파구도 분열 촉진시키지 못하였다. 전기영동상에서 하나의 주된 띠로 나타났으며 분자량은 Biogel P-300겔 여과에서 131,000, SDS 전기영동상에서는 125,000으로 나타났다. Subunit는 33,000과 30,000의 다른 폴리펩타이드로 tetramer로 추정된다. EDTA에 의해서 활성이 저해된 바지락 렉틴은 Ca^{++}과 Mn^{++}에 의하여 적혈구 응집력이 회복되었다. 또한 이 렉틴은 약 4.2 % 중성당을 함유한 당단백질임이 확인되었다.