

## Molecular Characterization of a New Lectin from the Marine Alga *Ulva pertusa*

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**Abstract** A new lectin, named UPL1, was purified from a green alga *Ulva pertusa* by an affinity chromatography on the bovine-thyroglobulin-Sepharose 4B column. The molecular mass of the algal lectin was about 23 kD by SDS-PAGE, and it specifically agglutinated rabbit erythrocytes. The hemagglutinating activity for rabbit erythrocytes could be inhibited by bovine thyroglobulin and *N*-acetyl-*D*-glucosamine. The lectin UPL1 required divalent cations for maintenance of its biological activity, and was heat-stable, and had higher activity within pH 6–8. The N-terminal amino acid sequence of the purified lectin was determined (P83209) and a set of degenerate primers were designed. The full-length cDNA of the lectin was cloned by rapid amplification of cDNA ends (RACE) method (AY433960). Sequence analysis of *upl1* indicated it was 1084 bp long, and encoded a premature protein of 203 amino acids. The N-terminal sequence of the mature UPL1 polypeptide started at amino acid 54 of the deduced sequence from the cDNA, indicating 53 amino acids lost due to posttranslational modification. The primary structure of the *Ulva pertusa* lectin did not show amino acid sequence similarity with known plant and animal lectins. Hence, this protein may be the paradigm of a novel lectin family.

**Key words** *Ulva pertusa*; lectin; purification; cloning

Lectins constitute a group of proteins or glycoproteins of non-immune origin, and they bind reversibly to carbohydrates and usually agglutinate cells or precipitate polysaccharides and glycoconjugates [1]. Due to their chemical properties, lectins attract a great deal of attention in the fields of immunology, cell biology, membrane structure, cancer research and genetic engineering, etc.. In recent years, there have been many reports on the antimicrobial and pesticidal activities of lectins, and the application of lectins in agriculture [2,3].

Lectins are present in a wide range of organisms from bacteria to animals, being present in all classes and families, although not in all the kinds and species [4]. Lectins from terrestrial plants and animals have been isolated, characterized and exploited extensively in many aspects of biochemistry and biomedicine. Compared with land plant lectins, the occurrence of lectins from marine algae,

however, was first reported in 1966 [5]. The first agglutinin was isolated and characterized from marine algae in 1977 [6,7]. Studies on marine alga lectins reveal a proteinaceous nature similar to those from land plants, but different in some of their properties. They have generally lower molecular masses than most land plant lectins and are more specific for complex oligosaccharides or glycoproteins. Further, most of marine algal lectins do not require divalent cations for their biological activity [8]. They occur mainly in monomeric form and have a high content of acidic amino acid, with isoelectric points from 4 to 6 [9–11]. Although several studies on lectins from marine algae have been reported [12–18], few lectins from algae have been characterized in detail [19], especially in the field of molecular biology. The amino acid sequence of the lectin from marine algae was first reported recently [20]. Up to now, there are only three full amino acid sequences of marine algae lectins reported from the marine red alga *Bryothamnion triquetrum* and *Hypnea japonica* [20,21]. Characterization of those lectin genes may help researchers to further understand the difference between land plant and marine alga lectins.

In this paper, we describe the purification, character-

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ization and full-length cDNA sequence of a new lectin from the marine green algae *Ulva pertusa*.

## Materials and Methods

### Materials

**Algae materials** The samples of *Ulva pertusa* were collected from Putian Bay, Fujian, China. They were rinsed with distilled water, and frozen in liquid nitrogen, and stored at  $-50^{\circ}\text{C}$  until use.

**Erythrocytes** Rabbit and chicken erythrocytes were obtained by venous puncture of healthy animals. Human blood groups A, B and O erythrocytes were collected from donors in Fuzhou Air Hospital, Fujian, China.

**Medium of chromatography** CNBr activated Sepharose 4B was purchased from Amersham Biotech Company, Sweden.

**Bacterial strains and vector** *E. coli* HB101 and pMD18T vector were provided by TaKaRa Biotechnology (Dalian) Co. Ltd., China.

**Primers** According to the N-terminal sequence, we designed the degenerate oligonucleotide primers, KSC1 and KSC2 for the 3' RACE of the lectin. Based on the 3' RACE results, we further generated sequence-specific primers, KSC3 and KSC4 for the 5' RACE (Fig. 1).

**Reagents and kit** UNIQ-10 Spin column total RNA isolation kit was purchased from Sangon Biological Engineering Technology and Service (Shanghai) Co., Ltd., China; SMART<sup>TM</sup> RACE cDNA amplification kit was provided by BD Bioscience Clontech Company, USA; Ultra-Clean<sup>TM</sup> 15 (DNA gel-purification kit) was provided by MO BIO Laboratories, Inc., USA. Other reagents were of

analytical grade.

### Purification of UPL1

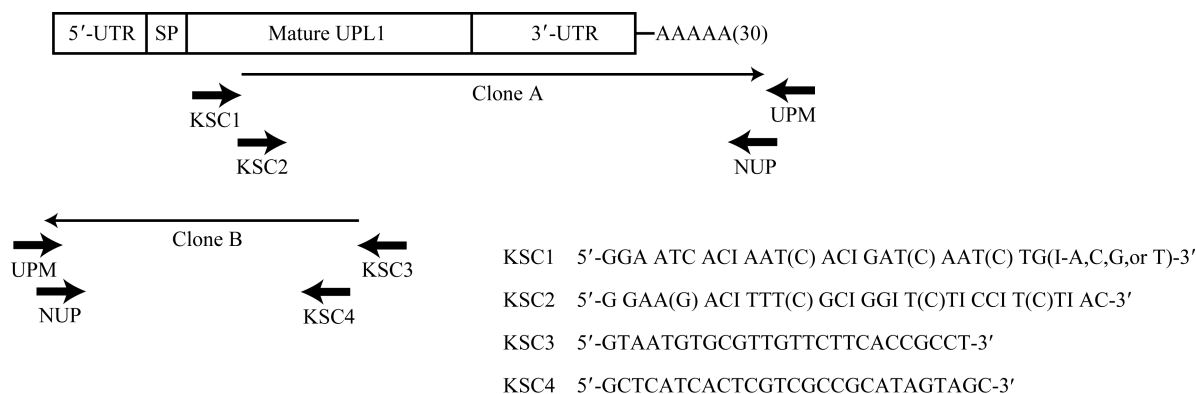
The frozen alga was macerated in domestic liquidizer with 0.015 M phosphate buffered saline (PBS, pH 7.0, containing 0.15 M NaCl) and centrifuged at 5000 rpm for 45 min at  $4^{\circ}\text{C}$  to remove particulate materials. The supernatant was transferred, and  $(\text{NH}_4)_2\text{SO}_4$  was slowly added to the final concentration of 80% saturation. The suspension was incubated at  $4^{\circ}\text{C}$  for 2 h, and precipitated by centrifugation at 5000 rpm for 45 min at  $4^{\circ}\text{C}$ . The precipitate was dialyzed against PBS. After centrifugation as above, the sample was loaded onto bovine-thyroglobulin-Sepharose 4B column (1.6 cm $\times$ 14 cm), equilibrated with the phosphate buffer at a flow rate 15 ml/h. The column was washed with the same buffer until the column effluent showed an absorbance less than 0.02 at 280 nm, and eluted with the phosphate buffer containing 1 M NaCl. Fractions of 3 ml were collected and assayed for hemagglutinating activity using rabbit erythrocytes. The active fractions were pooled, dialyzed extensively against distilled water, freeze-dried and then stored at  $-20^{\circ}\text{C}$  until use. The bovine-thyroglobulin-Sepharose 4B column was prepared according to the manufacturer's protocol.

### Homogeneity and molecular mass determination

The tests were performed as described by Costa *et al.* [9] using polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method.

### Hemagglutination and hemagglutination inhibition test

The hemagglutination and hemagglutination inhibition tests were performed as described by Costa *et al.* [9].



**Fig. 1 Schematic diagram of the cloning for the full-length cDNA of UPL1**  
 SP shows the signal peptide. The clones A and B show the 3' and 5' RACE cDNA clone of UPL1 respectively.

### Effect of EDTA, bivalent cations, temperature and pH on lectin stability

The tests were performed as described by Costa *et al.* [9].

### The neutral carbohydrate contents determination

Carbohydrate contents were determined by the phenol/sulphuric acid method [22], using glucose as standard.

### The N-terminal sequence determination

UPL1 was resolved by SDS-PAGE and then was electroblotted on PVDF membranes. After being stained with Coomassie brilliant blue, the target band was cut out from the PVDF membrane, and the N-terminal sequence was determined in the laboratory of protein chemistry of Hunan Normal University.

### Amplification of *upl1*

The 3' end of cDNA was amplified by nested PCR with first amplification using KSC1 and UMP, and followed one with KSC2 and NUP. The 5' end of cDNA was amplified by the same method as described above, using KSC3 and UMP first, then KSC4 and NUP (Fig. 1). The primers, UPM and NUP, were provided in the SMART™ RACE cDNA amplification kit.

The PCR products were gel-purified using UltraClean™ 15 according to the manufacturer's protocol. The purified PCR products were cloned into pMD18T vector, and screened with blue-white selection. The clones were confirmed by restriction digestion, and then sent for sequence analysis in BioAsia Company, Shanghai, China.

## Results

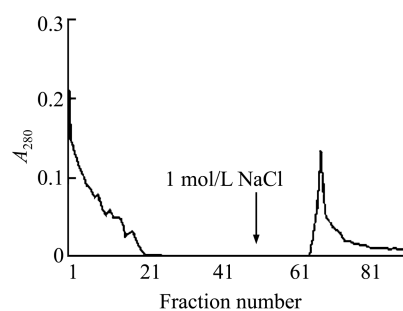
### Purification of UPL1

The lectin in *Ulva pertusa* extracts was purified by affinity chromatography on a column of the bovine-thyroglobulin-Sepharose 4B. UPL1 with full hemagglutinating activity in the aqueous extract was bound to the resin and could be eluted with 1 M NaCl (Fig. 2).

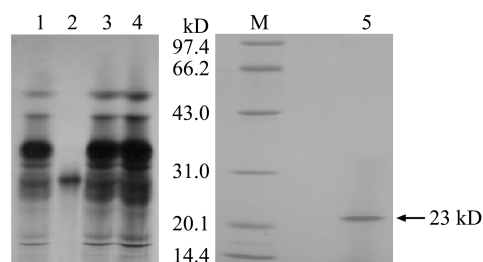
The homogeneity of the purified lectin was observed by a single band obtained by PAGE, while the purified lectin appeared as a single band with molecular weight of 23 kD on SDS-PAGE (Fig. 3), suggesting that the lectin could be a monomeric protein.

### Hemagglutination and hemagglutination inhibition test

The purified lectin only showed high specificity for rab-



**Fig. 2** Affinity chromatography of UPL1 on bovine-thyroglobulin-Sepharose 4B



**Fig. 3** PAGE and SDS-PAGE of UPL1

The electrophoresis pattern of PAGE is shown in the left, while that of SDS-PAGE is shown in the right. 1, 3 and 4, 80%  $(\text{NH}_4)_2\text{SO}_4$  fractionations; 2 and 5, purified lectins; M, protein molecular weight marker.

bit erythrocytes in all kinds of tested erythrocytes. The results of sugars inhibition tests using a large number of sugars and glycoproteins for UPL1 are presented in Table 1. The lectin did not show any inhibition by most of sugars test except *N*-acetyl-*D*-glucosamine. Of the glycoproteins tested, only bovine thyroglobulin was inhibitory requiring the minimum inhibitory concentration of 5.00 g/L.

### Effect of EDTA, bivalent cations, temperature and pH on lectin stability

To assess the requirement of the lectin for divalent cations in order to produce hemagglutination, titration of the lectin was performed in the presence of EDTA (Table 2). At a concentration of 0.005 M, EDTA totally abolished hemagglutination of rabbit erythrocytes. Addition of 0.01 M  $\text{CaCl}_2$  to the reaction medium restored the hemagglutination titer to the value  $2^6$ .  $\text{MnCl}_2$  or  $\text{ZnCl}_2$  at 0.01 M concentration also restored hemagglutination, but not in the same degree as that with  $\text{CaCl}_2$ .

The hemagglutination capacity of the lectin was not affected by exposure to 30–70 °C for 30 min. At 80 °C under the same condition the lectin still retained 50% activity.

Studies of the pH effects on the lectin revealed that the hemagglutination activity of the lectin was unaltered at pH 6–8, but lost its activity when buffer pH was below 3 or above 11.

**Table 1** Inhibition of hemagglutinating activity of UPL1 by sugars and glycoproteins

Sugars and glycoproteins	Initial concentration	Minimum inhibitory concentration
<i>D</i> -Mannose	0.5 M	—
<i>D</i> -Galactose	0.5 M	—
<i>D</i> -Fructose	0.1 M	—
Glucose	0.1 M	—
Sucrose	0.1 M	—
Maltose	0.1 M	—
Inulin	0.04 M	—
Rhamnose	0.04 M	—
Xylose	0.15 M	—
$\alpha$ -Lactose	0.15 M	—
<i>L</i> (-)-Fucose	0.2 M	—
<i>N</i> -Acetyl- <i>D</i> -galactosamine	0.2 M	—
<i>N</i> -Acetyl- <i>D</i> -glucosamine	0.2 M	0.0125 M
$\gamma$ -Globulin	27.00 g/L	—
Albumin bovine	40.00 g/L	—
Bovine thyroglobulin	40.00 g/L	5.00 g/L

“—” indicated the absence of inhibition.

### The neutral carbohydrate contents determination

The purified lectin was found to contain 1.2% neutral carbohydrate as determined by the phenol-sulphuric reaction.

### N-Terminal amino acid sequence

Twenty amino acids at N-terminus were determined as follows: GGITNTDNWETEFAGLPLTGA. The primers, KSC1 and KSC2, were synthesized according to the nucleotide sequence deduced from the underlined amino acids. This sequence has been submitted in SWISS-PROT protein knowledgebase with accession No. P83209.

### The full-length cDNA

Degenerate oligonucleotides corresponding to N-terminus of UPL1 were used to amplify the gene encoding the lectin. The full-length cDNA and its deduced amino acid sequence were obtained by analysis of 3' end and 5' end

**Table 2** Effect of EDTA and divalent cations on hemagglutination by UPL1

Solutions used as diluents	Titer
0.15 M NaCl	2 <sup>3</sup>
0.15 M NaCl + 0.005 M EDTA	2 <sup>0</sup>
0.15 M NaCl + 0.005 M EDTA + 0.01 M CaCl <sub>2</sub>	2 <sup>6</sup>
0.15 M NaCl + 0.005 M EDTA + 0.01 M MnCl <sub>2</sub>	2 <sup>2</sup>
0.15 M NaCl + 0.005 M EDTA + 0.01 M ZnCl <sub>2</sub>	2 <sup>3</sup>
0.15 M NaCl + 0.005 M EDTA + 0.01 M MgCl <sub>2</sub>	2 <sup>2</sup>

sequences of the cDNA clones (Fig. 4). The full-length cDNA with 1084 bp consists of 5' untranslated region (UTR) of 124 bp, an open reading frame (ORF) of 612 bp, and 3'-UTR of 348 bp including a poly(A) adenylation of 30 bp. The cDNA clone putatively encoded a protein of 203 amino acids. The N-terminal amino acid residues derived from amino acid sequencing were confirmed in the cDNA clone. The N-terminal sequence of the mature UPL1 polypeptide started at amino acid 54 of the deduced sequence from the cDNA, indicating 53 amino acids were lost due to posttranslational modification. The predicted molecular mass of the matured protein (17 kD) was significantly lower than the observed value, partially because it was a glycoprotein. The concentration of acidic amino acids is only 10.7% and the lectin does not contain cysteine. The predicted pI of the mature polypeptide was 8.74. The GC content preference at the third position of the triplet is 75%.

The primary structure of the *Ulva pertusa* lectin does not show amino acid sequence similarity to any known plant and animal lectins. Hence, this protein might be the paradigm of a novel lectin family.

### Discussion

Species of the green marine alga genus *Ulva* are widely distributed throughout the world. The attention of several research groups has been attracted to *Ulva* species, a potential source of exploitable lectin. For example, Gilboa-Garber *et al.* [23] have identified a fucose-binding lectin from *U. lactuca*. Other *Ulva* species have yielded lectins that are not inhibited by sample sugar, but are inhibited by more complex glycoproteins [24]. All lectins in those works have been examined in the crude or semi-purified extracts of the marine alga. The first description of purification of a lectin from the genus *Ulva* was not reported

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1 GAA TCC TGC ACG GGG CGT ATG CCG AGA TAT ACT GCG ACG ATA TCT CAA ATC GCC GAC AGG 60
61 TGG AGA CCA GAG CCT GAG GTC GAA AAC AGC CTA CTC CCG TCG CCG CTA CTA CAT TGT CGC 120
121 AAG CAT GAT CAA CAT CCT TCA TGT GAT TGC AGG CCT CGC GCT TGC TAG CGT GGG CGT TGA 180
1 M I N I L H V I A G L A L A S V G V D 19
181 CGC CCG CCA GGT AGG CGT CCG GGC TGA CGT GCT TCA CGC GGT GGA GAA CAC CAT TGA CAG 240
20 A R Q V G V G A D V L H A V E N T I D S 39
241 CAT TAC CGG CGT TGA GGC ATC ACA CAG CGC ACT CGA GGT TGG CCG TGG GAT TAC CAA TAC 300
40 I T G V E A S H S A L E V G G G I T N T 59
301 TGA CAA CTG GGA AAC GTT TGC CCG GCT GCC CCT TAC GGG TGC GAT CAA GGT GAA CGA TGG 360
60 D N W E T F A G L P L T G A I K V N D G 79
361 CAA GAG TGT TGT CCA TAT CTC AGC GTA GTT CCC CGA GGA CAG GCG GGG CAA GTA CAG CTA 420
80 N S V V H I S A Y F P E D R R G K Y S Y 99
421 CTA TGC GGC GAC GAG TGA TGA GCT CCA GAA GAC AGT GGT CTT CCT CTT TGT CGT TGA GGA 480
100 Y A A T S D E L Q K T V V F L F V V E D 119
481 CGA CCG CCT CCT CCT GGA GGC GGT GAA GAA GAA CGC ACA TTA CCC CGT CAC CAA CGG GAT 540
120 D G L L L Q A V K N N A H Y P V T N G M 139
541 GTA CCT CGC CTC ACA CCG GTA CTA CCC GAA GGA CAG CAA GTA CGA GGG CAT GGT CCG CCT 600
140 Y L A S H R Y Y P K D S K Y E G M V R L 159
601 GAT GGT GCA TGC CGA CCC CGC CAA GGC CGT CAT CTG GGA GTT TGT GAC GGT GGG CGG CAA 660
160 M V H A D P A K A V I W E F V T V G G K 179
661 GCA GTA CCT CAA GGT GAA GGA GAA CCG GGA CTA CAC AGC ACT CCA AAT AGC TCG CCA TCA 720
180 Q Y L K V K E N R D Y T A L Q I P R H H 199
721 CCC CCG ACC TGG ATA AGG CCA TGG CTG TAG AGG TCG TGC CAG TCG GTC CTG ATG GGC TTT 780
200 P R P G * 203
781 AGA CGA ACT GTG TCA AGG TGG TCG TGG AGG TTT GTT GCA TGT TGG CTG TAG GGT AGC GAA 840
841 GAT GGC CCT GTG ACG TAT GCT TCG CTG GGT GCT AGG AGC CTC ATG TTG CCG AAT TGT CAC 900
901 TGA TGA TTC CAG CTG GTT TTT GAT GGG AAT GCG GGA GCT GAA GGC TCC TTT TAC CAG CCC 960
961 AGA GGC TCT GCA AAG TCT AGG GGT GGG GTA GGT GTG AAT TTA AGC TGC AAG TAC CGT GAG 1020
1021 CTC GGG CAT GCA GCT CTT GAC TGA TGT AAT CTA TTG GAT TTC AAA AAA AAA AAA AAA 1080
1081 AAA A 1084

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**Fig. 4** The Nucleotide sequence of cDNA and deduced amino acid sequence for *upl1*

The N-terminal amino acid sequence is underlined. The asterisk indicates the stop codon. This cDNA sequence has been deposited in GenBank database with accession No. AY433960.

until 1996 [25]. Since then, other two lectins were purified from the genus *Ulva* [26,27]. The partial properties of those purified lectins were summarized in the Table 3.

Most characteristics of the lectin UPL1 are consistent with the previous descriptions about other *Ulva* lectins and other marine alga lectins. Like most lectins from *Ulva*

**Table 3** Properties of the lectins from genus *Ulva*

Material	<i>U. laetevirens</i> [25]	<i>U. lactuca</i> [26]	<i>U. pertusa</i> [27]
MW (SDS-PAGE)	18,900	17,120	Nd
MW (gel filtration)	30,300	8,370	11,047
pH stability	5.0 – 11.0	6.0 – 9.0	6.5 – 9.5
Heat stability	20 – 50	20 – 60	20 – 85
Inhibitor	Porcine stomach mucin Bovine submaxillary gland mucin Fucoïdan	Porcine stomach mucin Bovine submaxillary gland mucin Fucoïdan <i>L</i> -Fucose	Bovine thyroglobulin
Effect of EDTA	+	+	Nd

Nd, not detected; +, inhibition effect.

species, the *U. pertusa* lectin UPL1 required divalent cations for maintenance of its biological activity, because the addition of EDTA to reaction medium could affect the hemagglutinating activity. Furthermore, the inhibition of the hemagglutinating activity by glycoprotein was also observed in other lectins from *Ulva* species and other marine algae, such as *Bryothamnion triquetrum*, *B. seaforthii* [28], and *Amansia multifida* [9].

The lectin UPL1 and another one purified by Li *et al.* [27] were both from *U. pertusa*. But they have obvious difference in molecular weight and other slight differences were also observed in basic properties. There are remarkable differences in terms of their basic properties among the lectins isolated from the same alga species in red alga *Gracilaria domigensis* and *Gelidium pusillum* [29], and green alga *Ulva lactuca* [30]. The variations could be related to different phases in the life cycle of plant [30].

Based on the N-terminal amino acid sequence, the full-length cDNA sequence of UPL1 was cloned by PCR-RACE method, and sequenced. The full length of cDNA is 1084 bp, and it encodes a premature product of 203 amino acids. The N-terminal sequence of the mature UPL1 polypeptide started at amino acid 53 of the deduced sequence from the cDNA, indicating 53 amino acids were lost due to post-translational modification. The primary structure of the *Ulva pertusa* lectin does not show amino acid sequence similarity to known plant and animal lectins. Hence, this protein might be the paradigm of a novel lectin family. Apparently there is still much work to do on the sequences of algae lectins since only three amino acid sequences have been determined so far. More sequence studies will contribute to understanding their biochemistry and evolution differences between lectins present in land plants and those in marine algae.

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