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cDNA cloning and characterization of a rhamnose-binding lectin SUL-I from the toxopneustid sea urchin *Toxopneustes pileolus* venom

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ABSTRACT

The globiferous pedicellariae of the venomous sea urchin *Toxopneustes pileolus* contain several biologically active proteins. Among these, a galactose-binding lectin SUL-I isolated from the venom in the large globiferous pedicellariae shows several activities such as mitogenic, chemotactic, and cytotoxic activities through binding to the carbohydrate chains on the cells. We cloned cDNA encoding SUL-I by reverse transcription-PCR using the degenerate primers designed on the basis of the N-terminal amino acid sequence of the protein and expressed the recombinant SUL-I (rSUL-I) in *Escherichia coli* cells. The SUL-I gene contains an open reading frame of 927 nucleotides corresponding to 308 amino acid residues, including 24 residues of a putative signal sequence. The mature protein with 284 residues is composed of three homologous regions, each showing similarity with the carbohydrate-recognition domains of the rhamnose-binding lectins, which have been mostly found in fish eggs. While rSUL-I exhibited binding activity for several galactose-related sugars, the highest affinity was found for L-rhamnose among carbohydrates tested, confirming that SUL-I is a rhamnose-binding lectin. rSUL-I also showed hemagglutinating activity toward rabbit erythrocytes, indicating the existence of more than one carbohydrate-binding site to cross-link the carbohydrate chains on the cell surface, which may be closely related to its biological activities.

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1. Introduction

The venom obtained from the globiferous pedicellariae of the sea urchin *Toxopneustes pileolus* contains several biologically active proteins (Kimura et al., 1975; Nakagawa et al., 2003, 1991). Among these, the galactose-specific lectin SUL-I isolated from the venom of the large globiferous pedicellariae shows various activities such as chemotactic activity on guinea pig neutrophils and mitogenic activity on murine splenocytes by binding to the carbohydrate chains on target cells (Nakagawa et al., 1996; Takei and Nakagawa, 2006). N-terminal sequence analysis of SUL-I suggested that this lectin has some similarity with rhamnose-binding lectins (RBLs), majority of which have been isolated from fish eggs (Tateno, 2010). RBLs are also referred to as the sea urchin egg lectin (SUEL) family because of their homology with those found in the eggs of the sea urchin *Anthocidaris crasssispina*. Till date, the lectins having homology with SUEL have mostly been found in fish eggs, while some homologous proteins have also been found in mammals, e.g., mouse latrophilin-1, a putative G-protein-coupled receptor involved in synaptic function (Vakonakis et al., 2008).

In various organisms, lectins are known to play important roles in molecular and cellular recognition processes because of the vast diversity of the carbohydrate chain structures on their surface. Lectins are categorized into several families (Kilpatrick, 2002). Among these, two major groups are C-type lectins and galectins (S-type lectins) (Drickamer, 1988). C-type lectins were named owing to their Ca^{2+}-dependent carbohydrate-binding activity, and these lectins contain common carbohydrate-recognition domains (CRDs) composed of 110–130 amino acid residues. They are distributed in various organisms and are known to play important roles in various biological molecular recognition systems, including the immune system and cell adhesion processes (Drickamer, 1999). Some C-type lectins and C-type lectin-like proteins (Zelensky and Gready, 2005)

Abbreviations: CRD, carbohydrate-recognition domain; SUEL, sea urchin egg lectin; SUL-I, sea urchin lectin-I; PD, polyamidoamine dendrimer; RACE, rapid amplification of cDNA ends; RBL, rhamnose-binding lectin; TBS, Tris-buffered saline.

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have also been found abundantly in snake venoms (Igci and Demiralp, 2012). They contribute to the toxicity of the venom by binding to the carbohydrate chains on target cells of the victims. Therefore, it may be important to elucidate the structure and function of the lectins in animal venoms to understand their implications in toxicity. However, there is very limited knowledge concerning the lectins in animal venoms, particularly those from marine organisms.

In the present study, we have cloned SUL-I cDNA from the large globiferous pedicellariae of *T. pileolus* and expressed it in *Escherichia coli* cells to elucidate its structure and carbohydrate-binding properties. The results reveal its structural relationship with RBLs. The putative three-dimensional structure constructed by homology modeling using SUEL domain provides insights into its carbohydrate-recognition mechanism.

2. Materials and methods

2.1. Materials

Oligonucleotides and polyamidoamine dendrimers (PDs) with 64 amino surface groups (ethylenediamine core, generation 4.0, M.W. 14,214) (amino-PD) were purchased from Sigma–Aldrich. The plasmid vectors used in this study were as follows: pTAC-2 vector was obtained from DynaExpress and pET-3a expression vector was obtained from Novagen. Melibiose, lactose, and mannose were obtained from Wako Pure Chemicals (Osaka, Japan). Rabbit blood was obtained from Nippon Bio-Test Laboratories (Tokyo, Japan). The lactose-immobilized Cellulofine (lactose-Cellulofine) column was prepared by attaching lactose to Cellulofine gels (JNC Corp., Tokyo, Japan) using the cross-linking reagent divinyl sulfone (Sigma–Aldrich), as

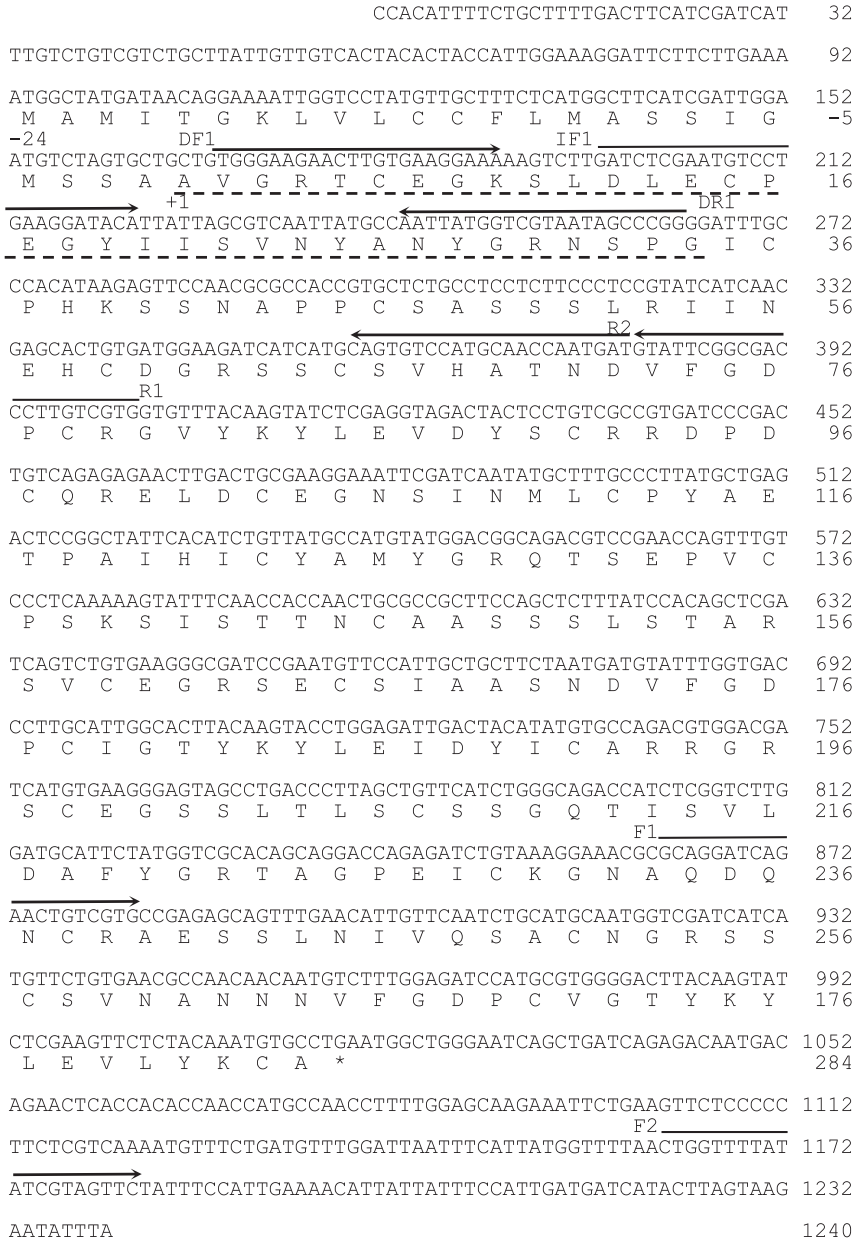

Fig. 1. The nucleotide and deduced amino acid sequences of SUL-I. The N-terminal amino acid sequence determined from the purified protein (Nakagawa et al., 2003) is indicated by a broken line. The N-terminal amino acid of mature protein is numbered as "+1." The primers used for PCR are indicated by horizontal arrows.

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