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A recombinant Sp185/333 protein from the purple sea urchin has multitasking binding activities towards certain microbes and PAMPs

Cheng Man Lun, Catherine S. Schrankel [1], Hung-Yen Chou [2], Sandro Sacchi [3], L. Courtney Smith *

The George Washington University, Biological Sciences, Science Engineering Hall, 800 22nd St. NW, Washington DC 20052, United States

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ABSTRACT

The purple sea urchin, *Strongylocentrotus purpuratus*, possesses a sophisticated innate immune system that responds to microbes effectively by swift expression of the highly diverse *Sp185/333* gene family. The Sp185/333 proteins are predicted to have anti-pathogen functions based on inducible gene expression and their significant sequence diversity. Sp185/333 proteins are all predicted to be intrinsically disordered and do not exhibit sequence similarities to other known proteins. To test the anti-pathogen hypothesis, a recombinant Sp185/333 protein, rSp0032, was evaluated and found to exhibit specific binding to marine *Vibrio diazotrophicus* and to *Saccharomyces cerevisiae*, but not to two *Bacillus* species. rSp0032 also binds to LPS, β-1,3-glucan and flagellin but not to peptidoglycan. rSp0032 binding to LPS can be competed by LPS, β-1,3-glucan and flagellin but not by peptidoglycan. We speculate that the predicted intrinsically disordered structure of rSp0032 may adapt to different conformations in binding to a limited number of PAMPs and pathogens. Given that rSp0032 binds to a range of targets, and that up to 260 different Sp185/333 proteins can be expressed per individual sea urchin, this family of immune response proteins may facilitate effective host protection against a broad array of potential pathogens encountered in the marine environment.

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1. Introduction

Echinoderms lack adaptive immune systems (reviewed in (Smith and Davidson, 1992; Gross et al., 1999)), yet they possess sophisticated innate immunity and produce diversified antigen recognition molecules for successful pathogen detection and clearance (Smith, 2012; Ghosh et al., 2010). The California purple sea urchin, *Strongylocentrotus purpuratus*, a member of the Echinoderm phylum, is phylogenetically positioned within the deuterostome lineage and is a sister group to the Chordate phylum (Wada and Satoh, 1994). Annotation of the sea urchin genome shows a complex repertoire of large immune gene families, including Toll-like receptors, nucleotide oligomerization domain-like receptors, scavenger receptor with cysteine-rich domains, lectins, and a unique gene family called *Sp185/333* (Smith, 2012; Ghosh et al., 2010; Hibino et al., 2006; Rast et al., 2006; Buckley and Rast, 2015; Sodergren et al., 2006). The *Sp185/333* transcripts make up a major category in immune activated sea urchin coelomocytes (immune cells), show significant upregulation in response to immune challenges from bacteria and several pathogen associated molecular patterns (PAMPs), and are not similar to any known sequence (Nair et al., 2005; Rast et al., 2000). The genes, messages and deduced proteins have intriguing sequence diversity, consistent with putative immune defense functions. The estimated size of the *Sp185/333* gene family is 50 (±10) genes per genome, and almost all genes are composed of two exons with a small intron (Smith, 2012; Terwilliger et al., 2007, 2006; Buckley et al., 2008a, 2008b; Buckley and Smith, 2007). The first exon encodes a hydrophobic

Abbreviations: CF, coelomic fluid; ESI-LTQ-MS/MS, electron spray ionization linear ion trap quadrupole tandem mass spectrometry; GlcNAC, N-acetyl glucosamine; IDP, intrinsically disordered protein; IDR, intrinsically disordered region; LPS, lipopolysaccharide; MurNAC, N-acetyl muramic acid; natSp185/333, native Sp185/333; NeuFITC, NeutrAvidin conjugated with fluorescein isothiocyanate; PAMPs, pathogen associated molecular patterns; PBS, phosphate buffered saline; PGN, peptidoglycan; RGD, arginine, glycine, aspartic acid; SRBCs, sheep red blood cells; wCF, whole.

* Corresponding author at: Science and Engineering Hall,Suite 6000,80022nd St NW,Washington DC 20052,Tel.:+ 202 994 9211,

E-mail address: csmith@gwu.edu (L.C. Smith).

[1] Present Address: Sunnybrook Health Sciences Centre and Department of Immunology, University of Toronto, Toronto, Canada.

[2] Present Address: Department of Microbiology and Immunology, Institute of Biomedical Sciences, George Washington University, Washington DC, United States.

[3] Present Address: Mother-Infant Department, Institute of Obstetrics and Gynecology, University Hospital of Modena, Modena, Italy.

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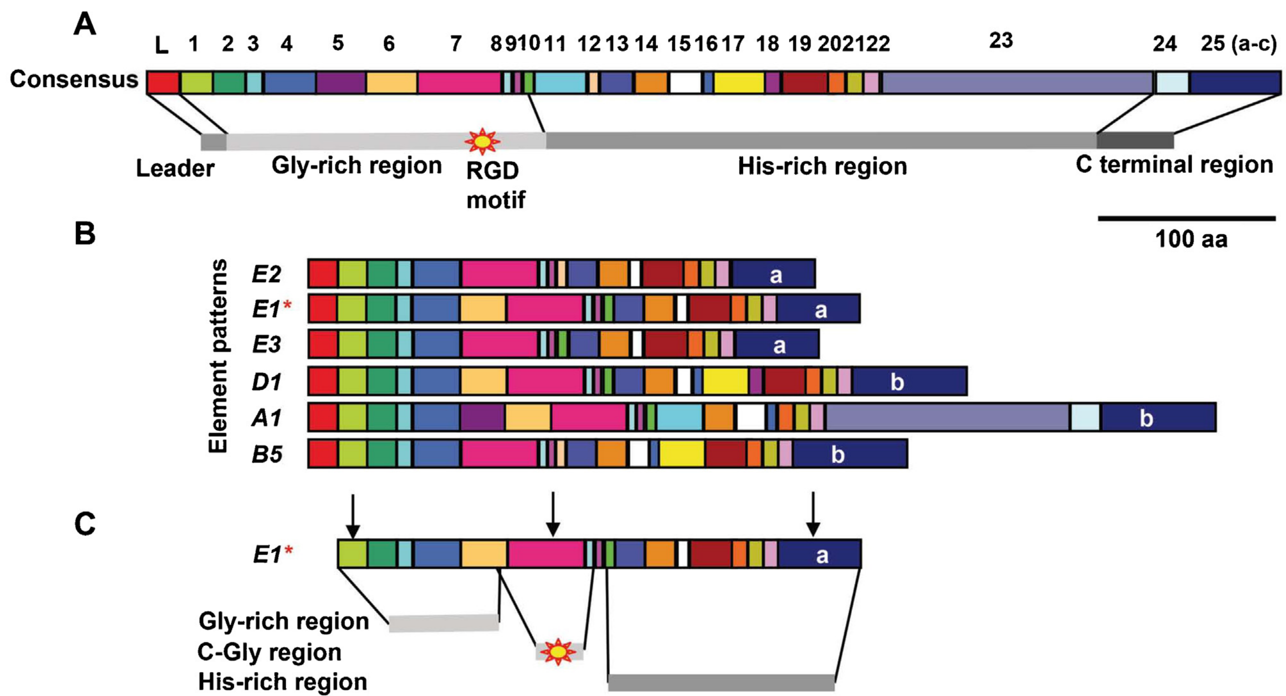

Fig. 1. Predicted Sp185/333 protein structure and diversity. (A) Sp185/333 proteins are composed of mosaics of elements, of which 25 are present in the consensus cDNA alignment that is shown (Terwilliger et al., 2007, 2006). Elements are blocks of sequence that are defined by gaps in the alignment and are illustrated as colored rectangles. Element 25 is subdivided into three sub-elements (a, b, and c) that are defined by three possible stop codons encoded by the genes. The predicted Sp185/333 proteins contain a leader (L), a gly-rich region (elements 1–9), a his-rich region (elements 10–23) and a C-terminal region (elements 24–25). An arginine, glycine, and aspartic acid (RGD) motif (sun symbol) with putative integrin binding function is present in element 7. (B) Mosaic combinations of different elements result in recognizable element patterns of the Sp185/333 proteins and provide significant diversity among the isoforms. A few element patterns are illustrated including *E1* that is the element pattern for rSp0032 (red asterisk). (C) The full-length rSp0032 structure without the leader plus the rGly-rich, rC-Gly and rHis-rich fragments are shown. Arrows indicate the positions of the peptides that were used to generate the three anti-Sp185/333 sera. Peptide sequences are available from (Table S1 in (Brockton et al., 2008) and Fig. 5B in (Dheilly et al., 2009)). Figures are modified from (Buckley and Smith, 2007). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

leader, which is likely cleaved during protein processing based on bioinformatic predictions. The second exon encodes the mature protein, which includes tandem and interspersed repeats. Optimal sequence alignments of *Sp185/333* genes (Buckley and Smith, 2007) and cDNAs (Terwilliger et al., 2006) require insertions of large artificial gaps that define a total of 25–27 recognizable blocks of sequence (depending on the alignment (Buckley and Smith, 2007)) known as *elements* (Fig. 1). Mosaic combinations of various elements plus as single nucleotide polymorphisms within the elements, small indels in the mRNAs, and several types of repeats, result in 51 *element patterns* that have been identified to date (Smith, 2012; Ghosh et al., 2010). The *Sp185/333* genes share element sequences, are all flanked by microsatellites, are tightly clustered, which may act in concert to promote genomic instability and sequence diversity of the family. Genetic diversity includes variations in gene copy number among individuals2 (Smith, 2012; Miller et al., 2010) and a wide range in gene sizes of 0.84 kb to 1.9 kb (Buckley and Smith, 2007). Furthermore, putative editing of the mRNAs that encode non-synonymous amino acids, introduce early stop codons and/or small indels that cause frame shifts resulting in missense sequences and truncated proteins (Smith, 2012; Ghosh et al., 2010; Terwilliger et al., 2007; Buckley et al., 2008b). Altered proteins that are the outcomes of RNA editing have been identified from the sea urchin coelomic fluid (CF) using proteomic methods (Dheilly et al., 2009). The proteome that can be generated by this system of ~50 genes can be up to 260 protein variants, which were identified in a single sea urchin (Dheilly et al., 2009).

The deduced Sp185/333 proteins show a common structure composed of an N-terminal, hydrophobic leader, a glycine (gly)-rich region containing an arginine-glycine-aspartic acid (RGD) motif, a histidine (his)-rich region and a C-terminal region (Terwilliger et al., 2006) (Fig. 1). Although the signal peptide suggests that the proteins are secreted, they are not detected in large quantities in the fluid phase of the CF. The Sp185/333 proteins are present within perinuclear vesicles of all types of the phagocyte class of coelomocytes as well as on the surface of small phagocytes (Ghosh et al., 2010; Dheilly et al., 2009, 2011; Brockton et al., 2008; Majeske et al., 2014). He185/333 proteins are also present in coelomocytes of the sea urchin, *Heliocidaris erythrogramma*, and are abundant in the trans Golgi network, on the internal membrane surfaces of vesicles and on the cell surface (Dheilly et al., 2011). However, the membrane association is unexpected because the proteins lack any predicted transmembrane regions or conserved sequences for glycophosphatidylinositol linkages. We have speculated that the RGD motif in the Sp185/333 proteins may be the basis for an association with membranes of phagocytes through binding to cell surface integrins (Ruoslahti, 1996; Whittaker et al., 2006), but this awaits confirmation.

Although there are no predictions of function for the Sp185/333 proteins based on the amino acid sequences, the diversity of the *Sp185/333* gene sequences, expression in response to immune challenge (Nair et al., 2005; Terwilliger et al., 2007, 2006) and variation in protein characteristics following pathogen exposure (Dheilly et al., 2009; Sherman et al., 2015) lead to the hypothesis that Sp185/333 proteins have immunological functions. Accordingly, we have tested native Sp185/333 proteins (natSp185/333 isolated from wCF) and a recombinant Sp185/333 protein, rSp0032 (with an E1 element pattern; see Fig. 1) for their binding activities towards bacteria, fungi, PAMPs, insect and mammalian cells. We developed assays based on Western blot, ELISA and flow cytometry to

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