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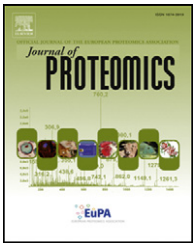

Mapping sea urchins tube feet proteome — A unique hydraulic mechano-sensory adhesive organ

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ABSTRACT

Marine organisms secrete adhesives for substrate attachment that to be effective require functional assembly underwater and displacement of water, ions, and weakly bound polyions that are ubiquitous in seawater. Therefore, understanding the characteristics of these protein/carbohydrate-based marine adhesives is imperative to decipher marine adhesion and also, to accelerate the development of new biomimetic underwater adhesives and anti-fouling agents. The present study, aims at mapping the proteome of the sea urchin *Paracentrotus lividus* adhesive organs using a combination of complementary protein separation techniques (1-D-nanoLC and 2-DE), databases and search algorithms. This strategy resulted in the identification of 328 non-redundant proteins, constituting the first comprehensive list of sea urchin tube feet proteins. Given the known importance of phosphorylation and glycosylation in marine adhesion, the 2DE proteome was re-analyzed with specific fluorescent stains for these two PTMs, resulting in the identification of 69 non-redundant proteins. The obtained results demonstrate that tube feet are unique mechano-sensory adhesive organs and highlight putative adhesive proteins, that although requiring further confirmation, constitute a step forward in the quest to decipher sea urchins temporary adhesion.

1. Introduction

Nowadays the adhesive technology is still challenged by the following requirements: (1) reliability of adhesive bonds, (2) environment-friendly glues, (3) application of small amounts of glue, (4) multiple attachments and detachments, and (5) efficient attachment to different surfaces in aqueous environments. Bio-inspired adhesives, particularly those from marine organisms, fulfill all these requirements and thus can provide solutions to many of the challenges faced by adhesive manufacturers. In fact, underwater attachment is a complex phenomenon, involving a number of sub-functions such as water displacement from the substratum, spreading of the adhesive, coupling to a variety of substrata, curing to stiffen and toughen the adhesive, and protection from microbial degradation [1]. Therefore, only a deep knowledge of marine adhesive functional properties and associated molecular mechanisms can lead to engineering innovative biomimetic adhesives for industrial and medical needs.

Among the chemical adhesive strategies employed by marine organisms, non-permanent adhesion is the least studied. Echinoderms are no exception and little is known on the molecular mechanisms that govern their ability to attach strongly to the substrate, and then detach easily and voluntarily from it, before reinitiating another attachment–detachment cycle [2,3]. Echinoderms possess a unique water–vascular system, also called ambulacral system, formed by canals and appendages, such as the tube feet. Although sea

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urchins possess many hundreds of tube feet spread all over their test, only those located at the oral surface are specialized in locomotion and temporary attachment (Fig. 1A). These adhesive appendages consist of a basal extensible cylinder, the stem, and an enlarged and flattened apical extremity, the disc (Fig. 1B). Together they form a functional unit, the stem allowing the tube foot to lengthen, flex and retract, whereas the disc makes contact with and adheres to the substratum [4]. Morphological studies have shown that when the substrate is suitable, the disc reorients its apical surface to be parallel to the substrate, makes contact with it and releases the content of adhesive granules contained inside specialized secretory cells, thus initiating the attachment process. Detachment of the tube foot is accomplished by the release of de-adhesive granules content by a second type of secretory cells, that are believed to secrete enzymes, which catalytic activity promotes breaking of the bonds established between the adhesive and the disc, thus leaving a circle of adhesive material (footprint) strongly attached to the substrate [3,5,6].

Although numerous individuals are necessary to obtain a sufficient amount of sea urchin adhesive material, tube feet footprints have recently been the object of a preliminary biochemical characterization [7]. Similarly to other marine adhesives, upon secretion sea urchin adhesive material becomes insoluble, thus requiring significant amounts of reducing agents and denaturants to be solubilized. Footprints were shown to contain inorganic residues (45.5%), proteins (6.4%), neutral sugars (1.2%), and lipids (2.5%) [7]. Although the importance of carbohydrates in temporary adhesion should not be overlooked, the current knowledge on marine adhesion focuses mainly on their adhesive proteins, since the best studied organisms (mussels and barnacles) attach permanently through the secretion of a cement that is mainly proteinaceous in nature [8,9]. The amino acid composition of sea urchin footprint protein fraction highlighted a bias toward 6 amino acids (glycine, alanine, valine, serine, threonine, and asparagine/aspartic acid), together with higher levels of proline (6.8%) and half-cystine (2.6%) than the average eukaryotic proteins. These traits are common to marine adhesives and are being pointed out as key factors for their high adhesive strength, cohesion and insolubility [3,8,10]. In addition, the 1-D profile of sea urchin footprints evidenced 13 proteins, of which 6 could be identified by MALDI-TOF/TOF MS conjugated with homology driven database search: alpha and beta tubulin, actin, and histones H2A, H2B, H3 and H4 [7]. Their presence is most likely due to remains of epidermal cellular material in the adhesive, although the possibility that these proteins actually belong to sea urchin adhesive bulk cannot be discarded. For the remaining unidentified proteins, the obtained MS/MS spectra were further processed by automated de novo-peptide sequencing, but again no homologies were found, suggesting that these proteins might be either novel or highly modified [7]. These results are not surprising given the paucity of sequenced marine organisms and hence marine adhesive proteins are often novel. In fact, so far only one barnacle cement protein was identified as a lysozyme homolog [8]. Recently, the carbohydrate moiety of the adhesive secretion of the sea star *Asterias rubens* was investigated using a set of 16 lectins, evidencing 2 footprint glycoproteins that enclose in their side chains galactose, N-acetylgalactosamine, fucose, and sialic acid residues that together with free sialylated proteoglycans are believed to provide both cohesion and adhesiveness through electrostatic interactions by the polar and hydrogen-bonding functional groups of their glycan chains [11]. So far there are no reports of the presence of phosphoproteins in echinoderm tube feet adhesive material. However, in other marine adhesive proteins, there are several reports of protein phosphorylation in serine residues (mussels, tube-worms and sea cucumbers) [12–14] that is believed to mediate adhesion to calcareous substrata [14], play a role in the condensation of the adhesive proteins in the adhesive cell secretory granules [12] and/or be involved in protein–protein cross-linking [15]. Interestingly, both sea urchin and sea star footprint materials present a bias toward serine and threonine residues (approximately 8%/residue) making their adhesive proteins prone to phosphorylation [6,7].

Therefore, the present study intends to complement the available information on the composition of sea urchins secreted adhesive (footprints) with a characterization of the proteome of their adhesive organs, in particular the oral tube feet discs, which contain the adhesive and de-adhesive secretory cells that enclose, respectively, the precursors of the adhesive and de-adhesive proteins. Yet, sea urchin tube feet discs do not contain exclusively these cells, but present a complex histological structure related with their adhesive function, being composed of an inner myomesothelium surrounding the water–vascular lumen (cavity that contains a

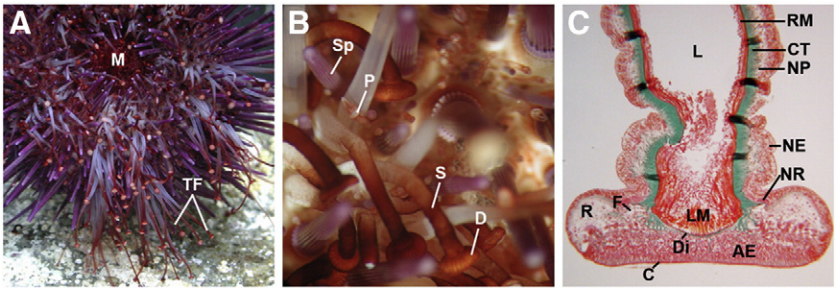

Fig. 1 – Oral surface of the sea urchin *Paracentrotus lividus* test (A) and enlarged view showing oral appendages (B). Histological structure of an oral tube feet (C). Abbreviations: AE, adhesive epidermis; Cu, cuticle; CT, connective tissue; D, disc; Di, diaphragm; F, frame; L, lumen; LM, levator muscle; M, mouth; NE, non-adhesive epidermis; NP, nerve plexus; NR, nerve ring; P, pedicillaria; R, rosette; RM, retractor muscle; S, stem; Sp, spine; TF, tube feet.