

CHARACTERIZATION OF ADHESIVE SECRETIONS FROM SEA STAR TUBE FEET

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Introduction

Sea stars can adhere strongly but temporarily to a variety of substrata using an adhesive secreted by a multitude of small appendages, the tube feet. This adhesive, because it acts in the presence of water, presents a strong potential for biomimetic applications in underwater construction (e.g., for the navy) or in the field of medicine and dentistry (Flammang et al., 2005; Waite et al., 2005).

Sea star tube feet are composed of a proximal cylinder, the stem and an enlarged and flattened apical extremity, the disc (Fig. 1A). When the tube foot makes physical contact with the substratum, as for instance during locomotion, two types of adhesive cells present in the disc epidermis release their sticky secretions through pores scattered on the disc surface (Fig. 1B), thus fastening the tube foot on the substratum. After the tube foot has detached, the adhesive material remains firmly bound to the substratum as a footprint (Flammang, 2006; Thomas & Hermans, 1985).

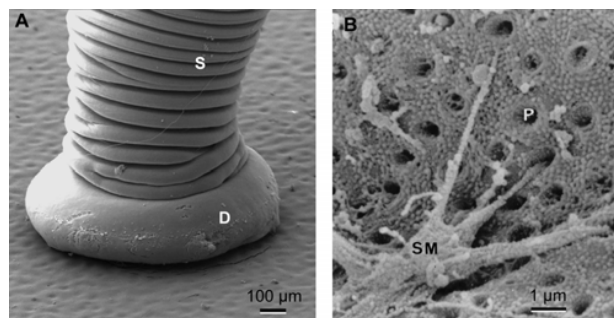


Figure 1. Tube foot adhesion and adhesive in the sea star *Asterias rubens* (from Flammang, 2006): **A:** SEM photograph of a tube foot attached to a textured polymer substratum; **B:** detailed view of the distal surface of the disc showing the release of adhesive secretion. D: disc, P: secretory pore, S: stem, SM: secreted adhesive material.

The material constituting the footprints is highly insoluble and, to date, its analysis has always required the complete hydrolysis of the footprints. Using these hydrolytic techniques, it was shown that the glue is made up mainly of proteins and carbohydrates (Flammang et al., 1998). It was not possible, however, to assess how many proteins were involved and whether the carbohydrate moiety was covalently attached to these proteins. Yet, to be

useful targets for biotechnological mimicry, sea star adhesive proteins should be purified and analysed separately. The present study aims at the extraction, separation and characterization of the different constituents of sea star footprints.

Materials and methods

Collection of footprint material

Individuals of the sea star *Asterias rubens* L. were allowed to walk across and/or attach to the bottom of clean glass Petri dishes filled with filtered sea water during 8 hours. The Petri dishes were thoroughly rinsed with ultra pure water and placed in a freeze dryer. The lyophilised footprint material was then scraped off using a glass knife and stored at -20°C (Flammang et al., 1998).

Characterization of the protein moiety

Proteins were extracted from footprint material according to the protocol developed by Kamino et al. (2000) to solubilize barnacle cement, with minor modifications. Briefly, 2 mg of lyophilised footprint material were submitted to two successive extractions. The first one was realised in presence of a buffer containing 6M guanidine hydrochloride. The second was performed in a buffer containing 7M guanidine hydrochloride, 20mM EDTA and 0.5M DTT. The proteins solubilized by the two extractions and the material remaining insoluble were suspended in SDS-PAGE sample buffer containing 6M urea and 2% β MSH and loaded onto a 8% polyacrylamide gel. After electrophoresis, the gel was stained with Coomassie G-250. The separated protein bands were manually excised and, after destaining, in-gel digested with trypsin. The resulting peptides were analysed by mass spectrometry using an HCT ultra ionic trap (Brucker).

Characterization of the carbohydrate moiety

Sixteen biotinylated lectins were applied either on tube foot histological sections or directly on footprints in order to highlight the presence of specific carbohydrate structures in adhesive cells and adhesive material. After labeling with streptavidin-texas red, the sections and footprints were observed using a Leica TCS 4D confocal laser scanning microscope.

The lectins which gave a positive signal in adhesive cells and/or footprints were used in blot analysis. Footprint proteins coming from the second extraction and separated by SDS-PAGE as described above were immobilized onto PVDF membranes. The membranes were incubated with the biotinylated lectins which were then labeled with streptavidin-HRP complex. Reactive bands were visualized by an Amersham ECL immunoblot detection system.

Results and Discussion

The two successive extractions allowed the solubilisation of about 25 protein bands ranging from 20 to 270 kDa in apparent molecular weight (Fig. 2). Protein concentration in the first extract was at least ten times lower than in the second extract. Among the proteins solubilised by the first treatment, all the minor protein bands (asterisks in Fig. 2) were successfully identified using mass spectrometry and corresponded to intracellular proteins (e.g., actin, tubulin, histones). These proteins probably come from contamination of footprint material by tube foot epidermal cells. On the other hand, the peptide sequences obtained with mass spectrometry for the two major protein bands did not correspond to any known sequence in protein and gene databases. The analysis of proteins bands solubilised after the second treatment with DTT revealed only unidentified proteins. Only one peptide of known sequence, similar to an actin peptide, was consistently detected among the peptides generated by the digestion of these different proteins. Based on their high concentration and unknown peptide sequences, we consider that protein bands resulting from the second extraction could correspond to the tube foot adhesive proteins. To simplify their description, these major protein bands were named sea star footprint proteins 1 to 5 (abbreviated Sfp 1-5). As for the material remaining insoluble after the two extractions, it was partially rendered soluble in the sample buffer after heating and contained the five Sfps.

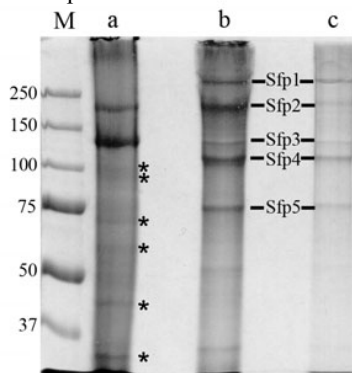


Figure 2. SDS-PAGE of major protein constituents from the footprints of *A. rubens*. **a**: proteins from the first extraction (the whole extract was loaded on the gel); **b**: proteins from the second extraction (only one tenth of the extract was loaded); **c**: insoluble fraction resuspended in SDS-PAGE sample buffer; **M**: molecular weight markers (kDa). Asterisks indicate some of the minor protein bands.

Among the sixteen lectins applied on tube feet sections, only seven (Con A, WGA, RCA, MAL II, GSL I, DBA and SBA) labelled the disc epidermis at the level of the adhesive cells (Fig. 3). Most of them labelled spherical structures which could correspond to secretory granules present in adhesive cells. On footprints, on the other hand, only Con A, WGA, RCA and DBA gave a positive signal (Fig. 3). These results indicate the presence of mannose (Con A), sialic acid (WGA), galactose (RCA) and N-acetylgalactosamine (DBA) residues in sea star adhesive secretions (Cummings, 1993; Monsigny et al., 1980; Gravel & Golaz, 1996). Three putative adhesive proteins were labelled on lectin blots: Sfp1 appeared to be linked to mannose (Con A) and sialic acid (Mal II) residues; Sfp2 appeared to be linked to mannose (Con A), N-acetylgalactosamine (DBA and GSL), sialic acid (MAL II) and galactose (RCA) residues; and Sfp3 appeared to be linked to N-acetylgalactosamine (DBA and GSL) residues. The difference of specificity in lectin binding to sialic acid (WGA on fresh footprints and MAL II on protein blots) could be explained by the different conformation of proteins in SDS-PAGE (denaturing conditions) and in footprints (native conditions).

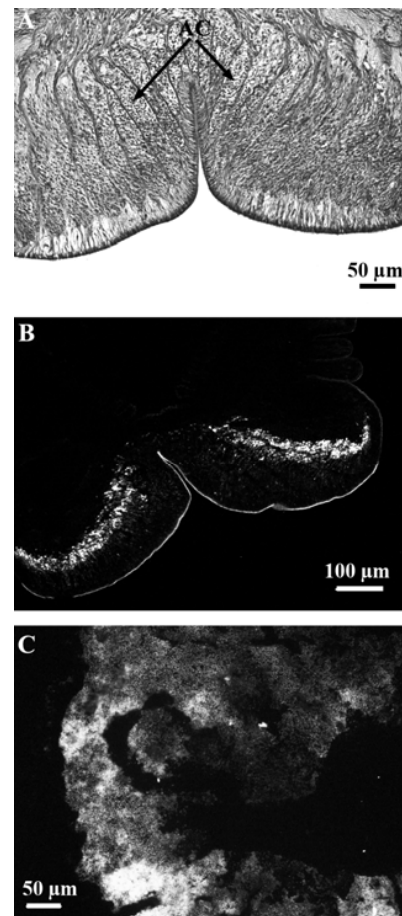


Figure 3. Carbohydrates in the adhesive secretions of *A. rubens*. **A,B**: Longitudinal sections through tube foot disc stained to show the tissue organization (**A**) and labelled with WGA (**B**). **C**: detail of a footprint labelled with RCA. AC: adhesive cells.

Conclusion

Taken in combination, the results suggest the co-occurrence of at least five different proteins in the adhesive footprints left by the tube feet of *A. rubens*. Among these proteins, two appear to be sialoglycoproteins and a third is a non-sialylated glycoprotein.

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