10 Adhesive Secretions in Echinoderms: An Overview

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10.1 Introduction

Members of the phylum Echinodermata are among the most familiar sea creatures, and representatives, such as the sea stars, have become virtually a symbol of sea life. The phylum contains about 7000 living species of relatively large invertebrates, all being exclusively marine and largely bottom-dwellers (Ruppert and Barnes 1994). There are five extant classes of echinoderms: the crinoids (sea lilies and feather stars), the asteroids (sea stars), the ophiuroids (brittle stars), the echinoids (sea urchins and sand dollars), and the holothuroids (sea cucumbers). The most striking characteristics of the group are the pentamerous radial symmetry and the presence of a unique system of coelomic canals and surface appendages composing the water-vascular system.

Echinoderms are also quite exceptional in the sense that most species belonging to this group use adhesive secretions extensively. Moreover, according to the species or to the developmental stage considered, different adhesive systems may be recognized. These include: (1) tube feet or podia, organs involved in attachment to the substratum, locomotion, food capture or burrowing; (2) larval adhesive organs allowing attachment of larvae during settlement and metamorphosis; and (3) Cuvierian tubules, sticky defence organs occurring in some holothuroid species. All these systems rely on different types of adhesion and therefore differ in the way they operate, in their structure and in the composition of their adhesive.

10.2 Tube Feet

Being exclusively benthic animals, echinoderms have activities and adaptations that are correlated with a relationship with the sea bottom. Most of these activities, such as attachment to the substratum, locomotion, handling of food, and burrow-building, rely on adhesive secretions allowing the animal to stick to or to manipulate a substratum. In post-metamorphic echinoderms,

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these adhesive secretions are always produced by specialized organs, the podia or tube feet. These are the external appendages of the water-vascular system and are also probably the most advanced hydraulic organs in the animal kingdom. Tube foot attachment is typically temporary adhesion. Indeed, although tube feet can adhere very strongly to the substratum, they are also able to detach easily and voluntarily from the substratum before reinitiating another attachment-detachment cycle (Thomas and Hermans 1985; Flammang 1996).

Tube feet have diversified into a wide variety of morphotypes, which were classified by Flammang (1996) into disc-ending, penicillate, knob-ending, lamellate, ramified, and digitate. In terms of adhesion, however, for practical considerations only disc-ending tube feet involved in attachment to the substratum and locomotion have been studied in detail. These tube feet consist of a basal hollow cylinder, the stem, and an enlarged and flattened apical extremity, the disc (Fig. 10.1A). The stem is extensible and flexible and allows the movements of the tube foot, whereas the disc makes contact with the substratum and releases the adhesive secretion (Fig. 10.1) (Flammang 1996).

Tube foot adhesive strength has been evaluated by measuring their tenacity, which is the adhesion force per unit area and is expressed in Pascals (Pa). Tenacity of single tube feet has been quantified in several species of asteroids and echinoids. The mean normal tenacity measured on a glass substratum is 170 kPa in *Asterias vulgaris* (Paine 1926), 198 kPa in *A. rubens* (Flammang and Walker 1997), and 59, 120 and 290 kPa in *Arbacia lixula*, *Sphaerechinus*

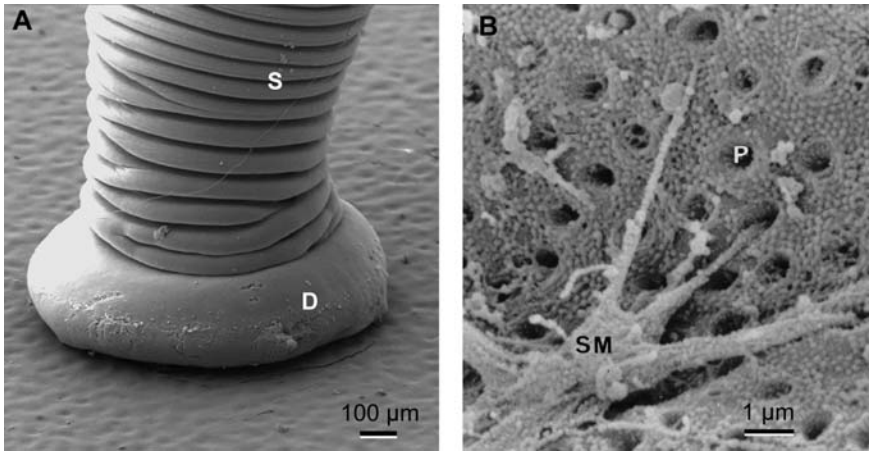

Fig. 10.1. Tube foot adhesion and adhesive in the asteroid *Asterias rubens*: (a) SEM photograph of a disc-ending tube foot attached to a textured polymer substratum (original); (b) detailed view of the distal surface of the disc showing the release of adhesive secretion (from Flammang et al. 1994). D disc, P secretory pore, S stem, SM secreted adhesive material

granularis, and *Paracentrotus lividus*, respectively (Flammang et al. 2005). On polymer substrata, the tube feet of *P. lividus* presented a mean tenacity of 340 and 140 kPa on polymethyl methacrylate (PMMA) and polypropylene, respectively; and the tube feet of *A. rubens* adhered to PMMA with a tenacity of 180 kPa (Santos et al. 2005a). All these values are in the same range as those observed in other marine invertebrates known to adhere very strongly to polymer and glass substrata, e.g., 170 and 230 kPa in limpets (Grenon and Walker 1981), 80 and 230 kPa in barnacles (Yule and Walker 1987), 120 and 750 kPa in mussels (Waite 2002), respectively). Moreover, it was recently demonstrated that asteroid and echinoid tube feet show increased adhesion on a rough substratum in comparison to its smooth counterpart (Santos et al. 2005a). This is because the disc adhesive surface is highly compliant, replicating the substratum profile. The increase in contact area between the disc and the substratum leads to a higher adhesion force (Santos et al. 2005a). Tube foot discs and their adhesive secretions therefore appear to be well-tailored to provide an efficient attachment to natural rocky substrata, allowing echinoderms to resist hydrodynamically generated forces.

The histological structure of the tube feet is remarkably constant for all echinoderm species. Their tissue stratification consists of four layers: an inner myomesothelium surrounding the water-vascular lumen, a connective tissue layer, a nerve plexus, and an outer epidermis covered externally by a cuticle (Flammang 1996). At the level of the tube foot tip, these tissue layers are specialized in adhesion and sensory perception: the connective tissue layer and the nerve plexus are thickened, and the epidermis is differentiated into a well-developed sensory-secretory epithelium. The latter comprises two types of secretory cells: non-ciliated secretory cells (NCS cells) enclosing large heterogeneous granules and ciliated secretory cells (CS cells) enclosing small homogeneous electron-dense granules (Fig. 10.2) (see Flammang 1996 for review). In some species, two types of NCS cells co-occur in the sensory-secretory epidermis. The study of the ultrastructure of the CS and NCS cells during a complete cycle of attachment-detachment of the tube foot in *A. rubens* (Fig. 10.2) demonstrated that they function as a duo-gland adhesive system as originally proposed by Hermans (1983), and in which NCS cells (types 1 and 2) release an adhesive secretion and CS cells a de-adhesive secretion (Flammang et al. 1994, 2005; Flammang 1996). The adhesive is present as a thin film between the tube foot cuticle and the substratum and, when detachment occurs, it takes place at the level of the outermost layer of the cuticle, the fuzzy coat, leaving the adhesive material strongly attached to the substratum as a footprint (Fig. 10.2) (Flammang 1996). In *A. rubens*, polyclonal antibodies have been raised against footprint material and were used to locate the origin of footprint constituents in the tube feet (Flammang et al. 1998a). Extensive immunoreactivity was detected in the secretory granules of both NCS1 and NCS2 cells, suggesting that their secretions make up together the bulk of the adhesive material. No immunoreactivity was detected in the secretory granules of CS cells and the only other structure strongly labelled

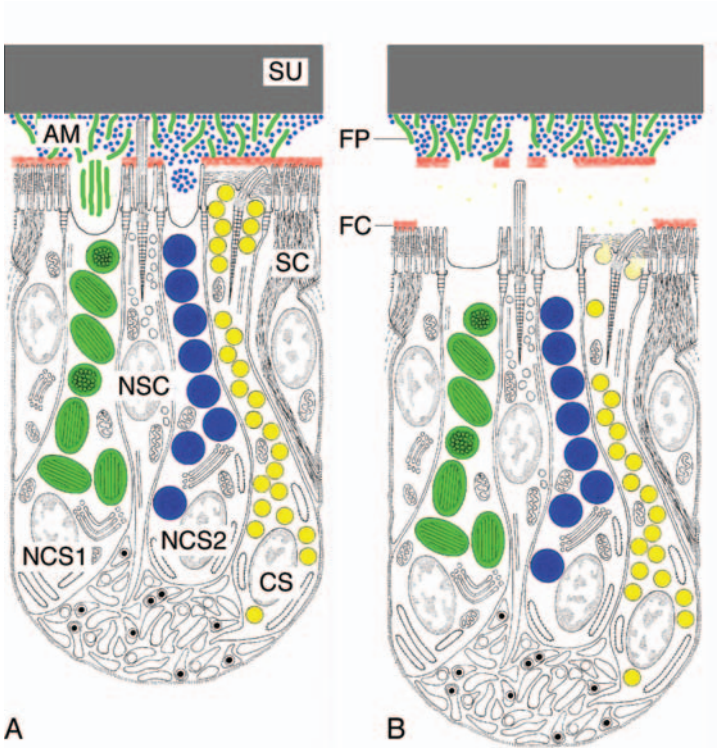

Fig. 10.2. Diagrammatic representation of the duo-gland model proposed for the attachment and detachment of a tube foot of the asteroid *Asterias rubens* using reconstructions of longitudinal sections through the disc epidermis (from Flammang et al. 1998a): (a) during attachment to the substratum, the two types of non-ciliated secretory cells (NCS1, *in green*, and NCS2, *in blue*) release some of their granules whose contents coalesce and mix to form the adhesive material; (b) during detachment from the substratum, ciliated secretory cells release their secretion (*in yellow*) which would function to jettison the fuzzy coat (*in red*) thereby allowing the podium to detach. The adhesive footprint left on the substratum after detachment thus comprises adhesive secretions and fuzzy coat material, but no de-adhesive secretion. AM adhesive material, CS ciliated secretory cell, FC cuticle fuzzy coat, FP footprint, NCS1 type 1 non-ciliated secretory cell, NCS2 type 2 non-ciliated secretory cell, NSC non-secretory ciliated cell, SC support cell, SU substratum

was the fuzzy coat. This pattern of immunoreactivity suggests that secretions of CS cells are not incorporated into the footprints, but instead might function enzymatically to jettison the fuzzy coat thereby allowing the tube foot to detach (Fig. 10.2B) (Flammang 1996; Flammang et al. 1998a).

Although the ultrastructure of the de-adhesive cell granules is remarkably constant from one echinoderm taxon to another, that of the adhesive cell granules varies extensively. The secretory granules of NCS cells are usually made up of at least two materials of different electron density which gives them a complex ultrastructure. Five broad categories can be recognized

(Flammang 1996): (1) homogeneous granules apparently made up of only one material, (2) heterogeneous granules in which two different materials are mixed in an irregular pattern (Fig. 10.3A), (3) dense-cored granules consisting of an electron-denser core surrounded by less dense material (Fig. 10.3B), (4) granules with a central filamentous bundle resembling granules of the previous group but in which the core is made up of a parallel arrangement of fibrils or rods (Fig. 10.3C), and (5) capped granules in which an electron-lucent material is covered, on one side, by a cap of electron-dense material (Fig. 10.3D). The significance of these ultrastructural differences between different echinoderm taxa is unknown at present. However, in asteroids, Engster and Brown (1972) pointed out a relationship between the internal organization of adhesive cell secretory granules and species habitat: asteroids confined to hard rocky substratum have complex granules enclosing a highly organized

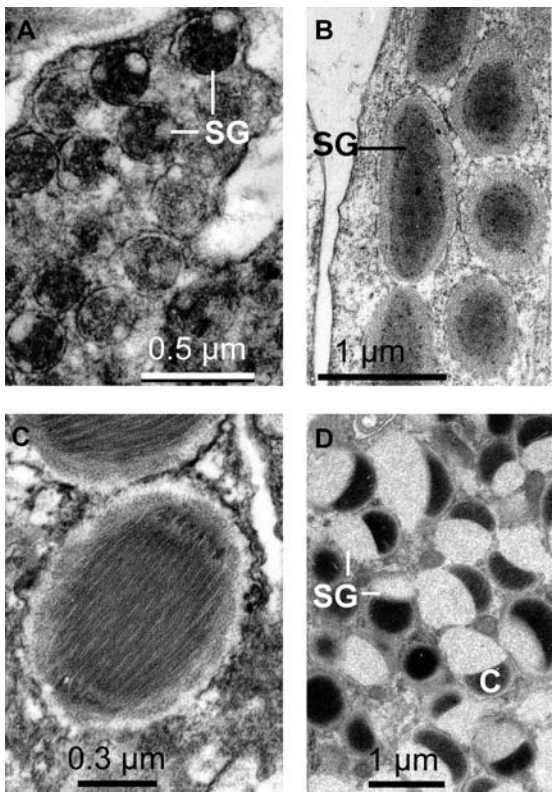

Fig. 10.3. Ultrastructure of the secretory granules of the adhesive cells from echinoderm tube feet (originals): (a) heterogeneous granules in the echinoid *Sphaerechinus granularis*; (b) dense-cored granules in the ophiuroid *Asteroxyx loveni*; (c) granules with a central filamentous bundle in the asteroid *Asterias rubens*; (b) capped granules in the holothuroid *Holothuria forskali*. C cap of electron-dense material, SG secretory granules

core whereas soft substratum dwelling species have granules of considerably simpler ultrastructure. They suggested that the different substructure of the adhesive cell granules would depend on the nature and composition of their contents that, in turn, could be related to the possible adhesive strength of the tube feet. In histochemistry, the adhesive cell secretory granules stain for both proteins and acid mucopolysaccharides, the former being predominant in some species and the latter in others (see Flammang 1996 for review).

In all echinoderm species investigated so far, after detachment of the tube foot, the adhesive secretion remains firmly bound to the substratum as a footprint. Although footprint diameter is easily measured after staining of the adhesive material (Flammang 1996), footprint thickness is difficult to estimate. Using an interference-optical profilometer, which generated three-dimensional images of the footprint surface, the mean maximum thickness of dry footprints was found to be 100 nm in the echinoid *P. lividus* and 230 nm in the asteroid *A. rubens* (Flammang et al. 2005). On the other hand, based on TEM observations, the thickness of fixed footprints in *A. rubens* ranges between 1 and 5 µm (Fig. 10.4C) (Flammang et al. 1994; Flammang 1996). Footprints appear as a foam-like material deposited as a thin layer on the substratum (Fig. 10.4A–C) (Thomas and Hermans 1985; Flammang 1996; Flammang et al. 1998a). This cellular aspect has been observed on fresh (Fig. 10.4A), freeze-dried (Fig. 10.4B), and fixed (Fig. 10.4C) footprints, and therefore is presumably not an artifact. In the asteroid *A. rubens*, the footprint adhesive material has the ultrastructure of a fibre-reinforced composite (Fig. 10.4D,E), which is able to fill out the very small surface irregularities – in the nanometer range – of the substratum (e.g., around the bacterium in Fig. 10.4E). The TEM study of the adhesive cells during tube foot attachment suggests that the electron-dense fibres present in the adhesive material derive directly from the rods described in their secretory granules (Figs. 10.2 and 10.3C). The chemical composition of the footprint material was analysed in *A. rubens*. Leaving inorganic residue apart, this material is made up mainly of proteins and carbohydrates, representing about 20 and 10% of the material's dry weight, respectively (Flammang et al. 1998a). The protein moiety contains significant amounts of both charged (especially acidic) and uncharged polar residues. Moreover, it has somewhat higher levels of glycine, proline, isoleucine and cysteine than the average eukaryotic protein (Table 10.1), this latter amino acid being presumably involved in intermolecular disulphide bonds reinforcing the cohesive strength of the adhesive (Flammang et al. 1998a). The carbohydrate moiety is also acidic, comprising both uronic acids and sulfate groups. So far, *A. rubens* is the only species in which the tube foot adhesive has been studied biochemically and nothing is known on other echinoderm species. Regarding the asteroids, however, a comparative immunohistochemical study of the tube feet from 14 species representing 5 orders and 10 families revealed that the adhesives of all these species are at least partly related, and this independently of the taxon considered, of the species habitat, of the tube foot morphotype or function, and of the adhesive cell secretory granule ultrastructure (Santos et al. 2005b).

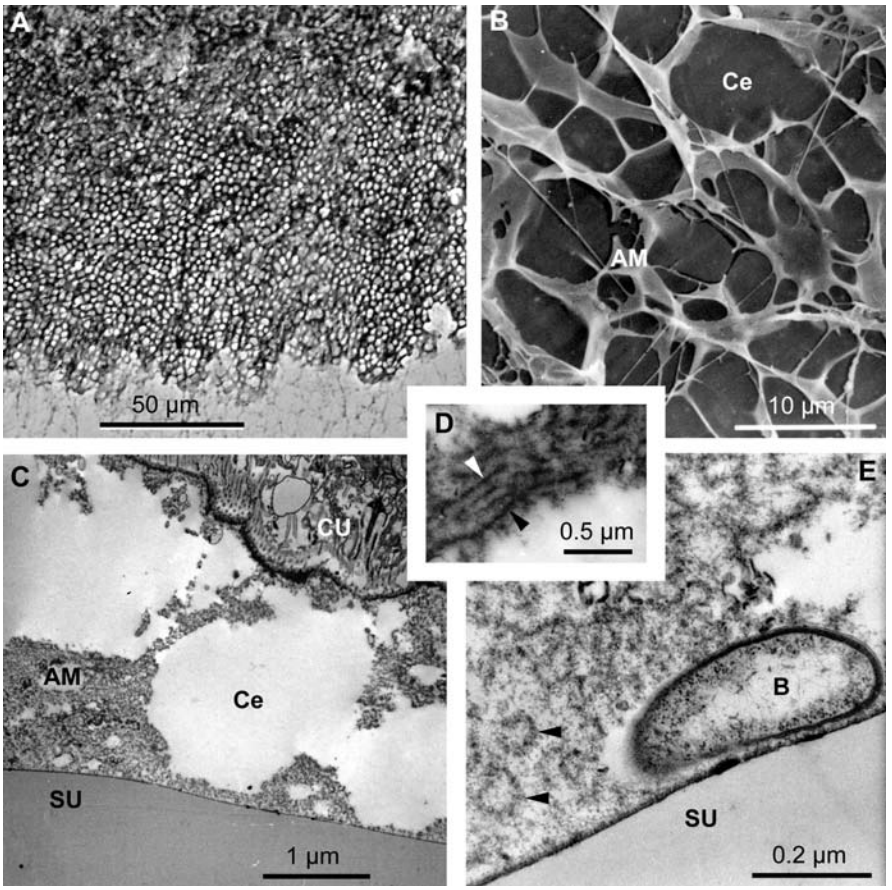

Fig. 10.4. Adhesive footprints and attached tube feet of the asteroid *Asterias rubens* (originals): (a) light microscopy photograph of a fresh footprint stained with a 0.05% aqueous solution of Crystal Violet; (b) detail of a freeze-dried footprint in SEM; (c) TEM photograph of a tube foot disc bond to the substratum by adhesive material; (d,e) details of the ultrastructure of the adhesive material (*arrowheads* indicate electron-dense fibre-like structures). AM adhesive material, B bacterium surrounded by the adhesive material, Ce cell in the foam-like adhesive, CU tube foot disc cuticle, SU substratum

10.3 Larval Adhesive Organs

For most echinoderms, metamorphosis transforms a bilaterally symmetrical and pelagic larva into a radially symmetrical and benthic postmetamorphic individual. Settlement always takes place during the so-called perimetamorphic period (Gosselin and Jangoux 1998; Haesaerts et al. 2003), but either before or after the metamorphic stage according to the class considered (Strathmann 1978). In both cases, adhesive organs attach either the competent

Table 10.1. Amino acid composition of the adhesive secretion from the tube feet of the sea star *Asterias rubens* (values in residues per thousand; from Flammang et al. 1998a)

Amino acid	
ASX	118
THR	78
SER	76
GLX	102
PRO	61
GLY	97
ALA	62
CYS/2	32
VAL	67
MET	17
ILE	45
LEU	61
TYR	27
PHE	38
HIS	56
LYS	21
ARG	41

larva or the postlarva to the substratum during settlement. In three of the five extant echinoderm classes, these organs are the tube feet, viz. the five primary tube feet of competent echinoplutei in echinoids, the five primary tentacles (and, for some species, two posterior tube feet) of pentactulae in holothuroids, the five primary tube feet and the five first pairs of tube feet of ophiuroid postlarvae (Strathmann 1978). These tube feet are similar in structure and function to tube feet of adults (Cameron and Fankboner 1984; Flammang et al. 1998b). Larval adhesive organs of crinoids and asteroids are, on the other hand, unique and have no equivalent in the postmetamorphic stage (Strathmann 1978).

The perimetamorphic period of crinoids comprises three stages: the doliolaria (free swimming larval stage), the cystidean (attached metamorphic stage), and the pentacrinoid stages (attached postlarval stage) (Mladenov and Chia 1983; Lahaye and Jangoux 1987; Nakano et al. 2003). Competent doliolariae are small barrel-shaped larvae. They possess an attachment complex at their anterior end which consists of a ciliary cap surrounding an apical tuft of elongated cilia and a ventrally located and slightly depressed adhesive pit (Fig. 10.5A,B). The ultrastructure of this attachment complex has been studied in comatulids (Chia et al. 1986; Jangoux and Lahaye 1990). It is strictly epidermal and made up of elongated ciliated cells associated with a thick basiepider-

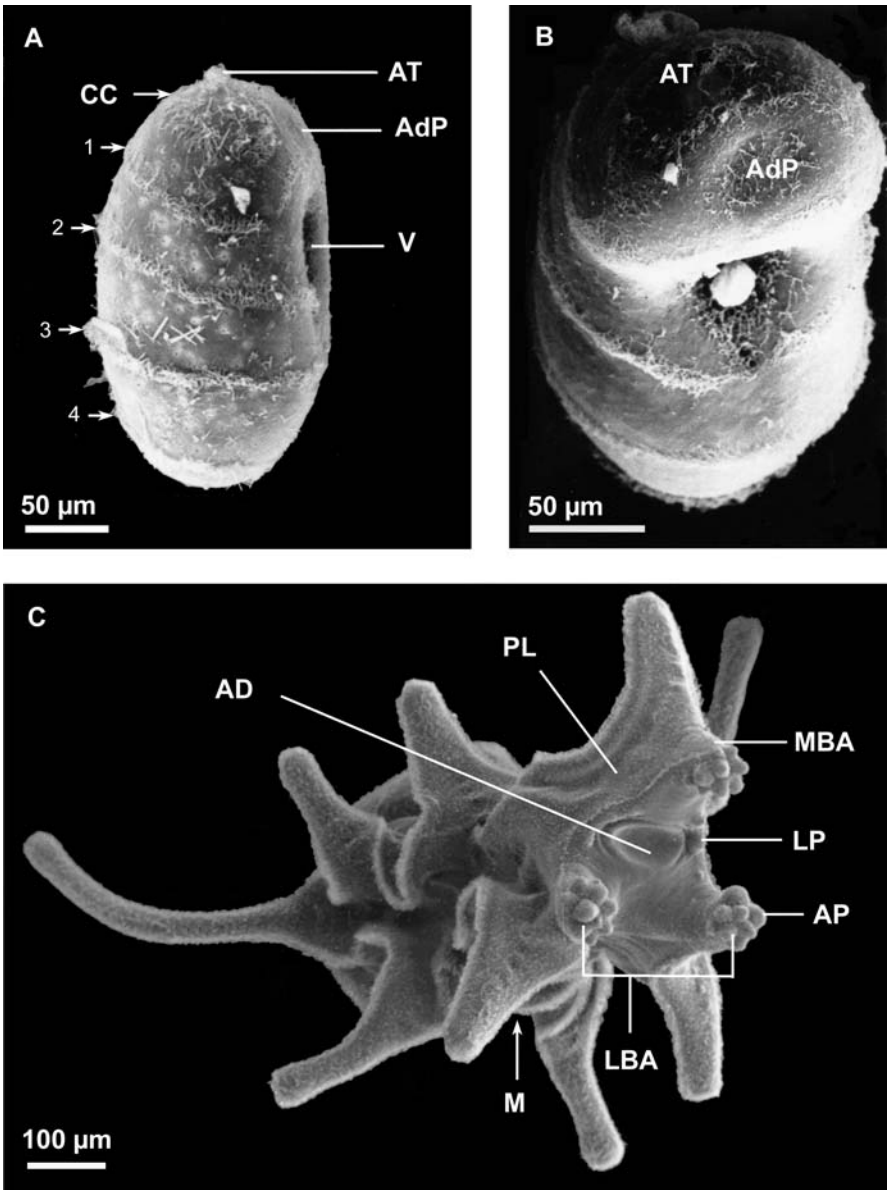

Fig. 10.5. Larval adhesive organs of crinoids and asteroids. SEM photographs of: (a) the doliolaria larva of *Antedon bifida*; (b) its anterior adhesive pit (from Lahaye 1987); (c) the brachiolaria larva of *Asterias rubens* (from Flammang et al. 2005). AD adhesive disc, AdP adhesive pit, AP apical papilla, AT apical tuft, CC ciliary cap, LBA lateral brachiolar arm, LP lateral papilla, M mouth, MBA median brachiolar arm, PL preoral lobe, V vestibule, 1–4 ciliary bands

mal nerve plexus. The four cell types forming the complex are sensory cells, covering cells and two types of secretory cells. Sensory cells and secretory cells of the first type occur exclusively in the ciliary cap. The former bear a long vibratile cilium whereas the latter are filled with secretory granules, which contain a flocculent mucopolysaccharidic material. Secretory cells of the second type are restricted to the adhesive pit where they are the most abundant cell type. These cells are filled with secretory granules with an electron dense fibrillar proteinaceous content. At the beginning of the settlement phase, the doliolaria becomes demersal and brushes the substratum with its apical tuft (sensory structure) (Mladenov and Chia 1983; Lahaye and Jangoux 1988). This implies the occurrence of a mechanism allowing the larva to combine loose adhesion to the substratum with movement. This transitory adhesion is achieved by the combined action of the secretory cells of the ciliary cap that produce a thin mucous film retaining the larva at the water-substratum interface, and of the covering cells whose cilia beat in this mucus (Jangoux and Lahaye 1990; Flammang 1996). When reaching a suitable site, the larva stops moving and turns itself round to have its body directed obliquely (the adhesive pit facing the substratum). It then becomes permanently fixed and transforms into a cystidean larva (Mladenov and Chia 1983; Lahaye and Jangoux 1988). Permanent adhesion starts with the release of the proteinaceous cement by the secretory cells of the adhesive pit and continues during both cystidean and pentacrinoid stages (Chia et al. 1986; Jangoux and Lahaye 1990). After development of the cirri during this last stage, the juvenile detaches from its cemented stalk (Lahaye and Jangoux 1987).

Competent larvae in asteroids are called brachiolariae because they possess a specialized attachment complex on their anterior part comprising three brachiolar arms and an adhesive disc (Fig. 10.5C) (Barker 1978; Haesaerts et al. 2003). Brachiolar arms are hollow tubular structures occupied by an extension of the larval anterior coelom. Their histological organisation comprises four tissue layers: an inner myomesothelium, a connective tissue layer, a subepidermal nerve plexus, and an outer epidermis. Each brachiolar arm is tipped by several sensory-secretory areas named papillae, where both the epidermis and the nerve plexus are greatly thickened (Fig. 10.6A). The papillary epidermis encompasses two types of secretory cells (adhesive and de-adhesive cells), sensory cells, and support cells (Barker 1978; Haesaerts et al. 2005a). Adhesive cells bear an apical cilium and contain large ovoid granules that enclose an electron-dense heterogeneous material staining histochemically as neutral mucopolysaccharides. De-adhesive cells bear a sub-cuticular cilium and are filled with small granules containing an homogeneous electron-dense material. The adhesive disc is a round, concave structure lying between the brachiolar arms. It is an epidermal structure composed of two main cell types (Fig. 10.6B): ciliated cement-secreting cells and support cells (Barker 1978; Haesaerts et al. 2005a). The former are full of large secretory granules enclosing a fibrous proteinaceous content of woven aspect. When exploring the substratum, the competent larva orients itself ventral side down and successively attaches and

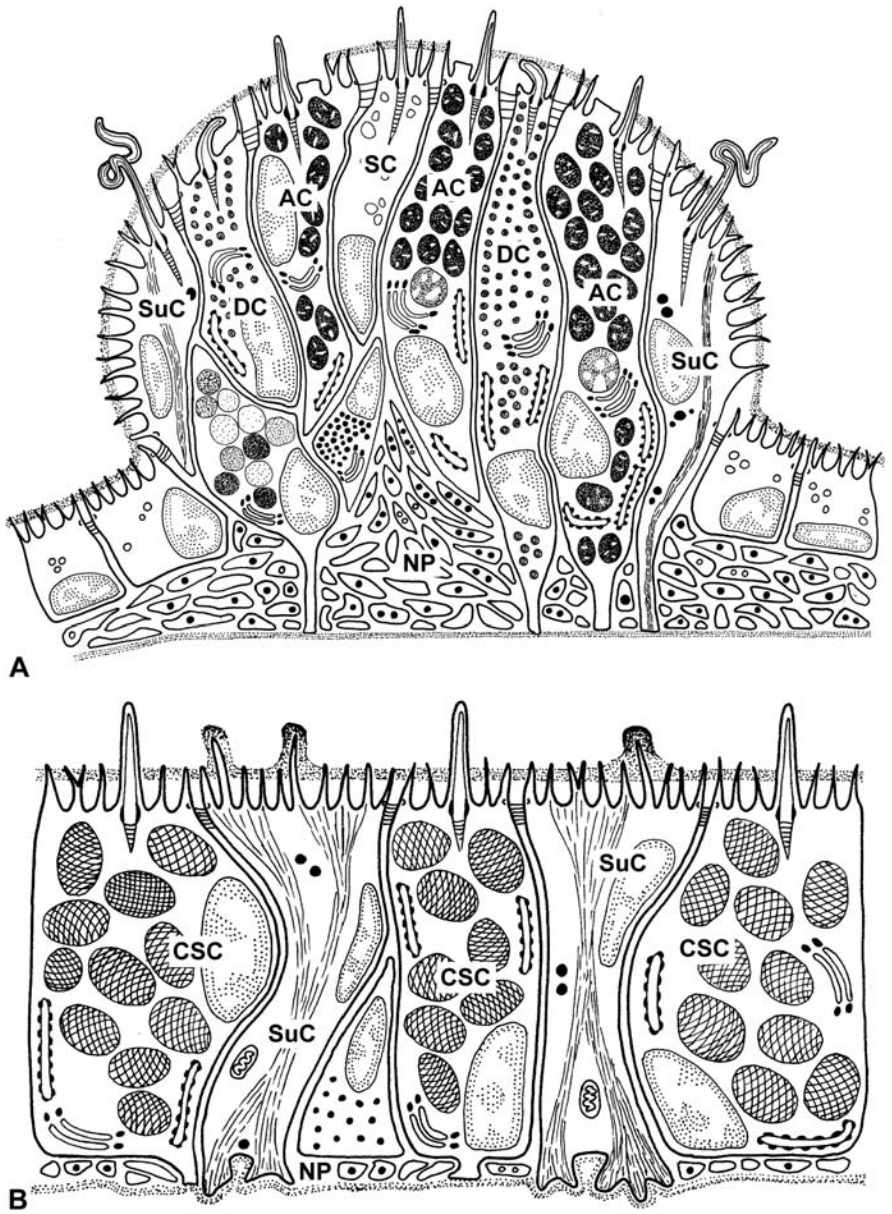

Fig. 10.6. Adhesive organs of the brachiolaria larva of the asteroid *Asterias rubens*. Schematic drawings of: (a) a longitudinal section through the papilla epidermis of a brachiolar arm; (b) a section through the adhesive disc epidermis (from Haesaerts et al. 2005a). AC adhesive cell, CSC cement secreting cell, DC de-adhesive cell, NP nerve plexus, SC sensory cell, SuC support cell

detaches its brachiolar arms (Barker 1978; Strathmann 1978; Haesaerts et al. 2003). Papillae, when making contact with the substratum, are responsible for sensory testing and temporary adhesion. Like adult tube feet, they function as a duo-gland system (Hermans 1983; Flammang 1996; Haesaerts et al. 2005a). In addition, the contents of brachiolar arm adhesive cells cross-react with antibodies raised against tube foot adhesive of *A. rubens*, indicating that temporary adhesives from larvae and adults are related to each other and probably share identical molecules, or, at least, identical epitopes on their constituents (Haesaerts et al. 2005a). Once the larva has found a suitable site for metamorphosis, brachiolar arms are gradually splayed out, enabling the disc to release its cement (Barker 1978; Haesaerts et al. 2003). This attaches the larva permanently to the substratum and marks the onset of the metamorphic stage. During this stage, tube feet become functional and ultimately help the newly formed postlarva to detach from the cemented disc (Haesaerts et al. 2003). In the species *Asterina gibbosa*, a turbulent channel flow apparatus has been used to evaluate the attachment strength of the different developmental stages (Haesaerts et al. 2005b). Using this technique, the nominal wall shear stresses needed to dislodge temporarily attached individuals are similar: about 1 Pa for brachiolariae attached by the arms and 7 Pa for postmetamorphic individuals attached by tube feet. On the other hand, a nominal wall shear stress of about 40 Pa is needed to detach metamorphic individuals permanently attached by the disc, showing the higher adhesive strength of the cement.

10.4 Cuvierian Tubules

Cuvierian tubules are peculiar organs found in several species of holothuroids (sea cucumbers), all belonging exclusively to the family Holothuriidae. Tubules (Fig. 10.7A) occurring in holothuroids of the genera *Bohadschia*, *Holothuria* and *Pearsonothuria* are expelled as sticky white threads that function as a defence mechanism against predators (Hamel and Mercier 2000; Flammang et al. 2002). Cuvierian tubule adhesion is a typical example of instantaneous adhesion, adhesion being achieved in a matter of seconds (less than 10 s; Zahn et al. 1973).

Cuvierian tubules occur in great numbers (between 200 and 600 in *H. forskali*; VandenSpiegel and Jangoux 1987) in the posterior part of the body cavity of the holothuroid. Proximally they are attached to the basal part of the left respiratory tree and their distal, blind end floats freely in the coelomic fluid. When disturbed, the sea cucumber directs its aboral end toward the stimulating source and undergoes a general body contraction. The anus opens, the wall of the cloaca tears, and the free ends of a few tubules (usually 10 to 20 in *H. forskali*; VandenSpiegel and Jangoux 1987), together with coelomic fluid, are expelled through the tear and the anus. As water from the respiratory tree is forcefully injected into their lumen, the emitted tubules elongate up to 20 times their

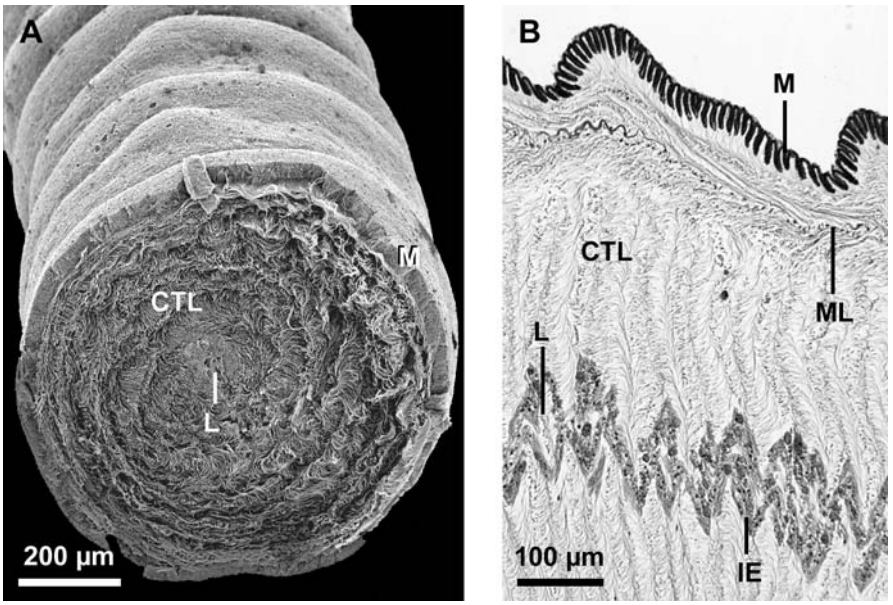

Fig. 10.7. Morphology of the Cuvierian tubules of *Holothuria impatiens* (from Flammang et al. 2005): (a) SEM photograph of a transversally-sectioned tubule; (b) longitudinal histological section showing the arrangement of the tissue layers. CTL connective tissue layer, IE inner epithelium, L lumen, ML muscle layer, M mesothelium

original length (VandenSpiegel and Jangoux 1987). Upon contact with any surface, the elongated tubules instantly become sticky. The adhesiveness of Cuvierian tubules combined with their tensile properties make them very efficient for entangling and immobilizing most potential predators (VandenSpiegel and Jangoux 1987; Hamel and Mercier 2000). Finally, the expelled tubules autotomize at their attachment point on the left respiratory tree and are left behind as the holothuroid crawls away (VandenSpiegel and Jangoux 1987). After expulsion and autotomy, Cuvierian tubules are readily regenerated. Cuvierian tubules thus constitute an efficient defensive mechanism. Their large number, sparing use and regeneration dynamics make them a formidable line of defense (Hamel and Mercier 2000; VandenSpiegel et al. 2000).

Cuvierian tubule adhesive strength on glass has been measured in seven species of sea cucumbers belonging to the genera *Bohadschia*, *Holothuria* and *Pearsonothuria* (Flammang et al. 2002). The mean normal tenacity observed varies from about 30 to 135 kPa. These values fall within the range of adhesive strengths described for marine organisms. They lie, however, among the lowest observed values (Flammang 2003). Tubule tenacity is influenced by the nature of the substratum: tubules adhere more strongly to polar than to non-polar substrata, indicating the importance of polar interactions in adhesion (Flammang et al. 2002).

Cuvierian tubules consist of, from the inside to the outside, an epithelium surrounding the narrow lumen, a thick connective tissue layer, and a mesothelium lining the surface of the tubule that is exposed to the coelomic cavity (Fig. 10.7). The mesothelium is responsible for adhesion. In quiescent tubules, it is a pseudostratified epithelium made up of two superposed cell layers – an outer layer of peritoneocytes and an inner layer of granular cells which is highly folded along the long axis of the tubule (Fig. 10.7B). Granular cells are filled with densely packed membrane-bound granules enclosing a proteinaceous material (Endean 1957; VandenSpiegel and Jangoux 1987). During elongation, the structure of the mesothelium is modified: the protective outer layer of peritoneocytes disintegrates and the granular cell layer, now unfolded, thus becomes outermost on the tubule. Granular cells empty the contents of their granules when the elongated tubule comes into contact with a surface, resulting in adhesion (VandenSpiegel and Jangoux 1987; De Moor et al. 2003).

In *H. forskali*, tubule print material – i.e., the secreted adhesive left on the substratum after mechanical detachment of the tubule – is composed of 60% protein and 40% neutral carbohydrate (De Moor et al. 2003). The proteinic nature of the adhesive material is confirmed by the observation that proteolytic enzymes reduce the adhesive strength of Cuvierian tubules in *H. forskali* (Zahn et al. 1973). The amino acid compositions of the protein fraction in *H. forskali*, *H. leucospilota*, *B. subrubra*, and *P. graeffei* indicate that their adhesives are closely related (Tables 10.2 and 10.3). All are rich in small side-chain amino acids, especially glycine, and in charged and polar amino acids. Only a small fraction of the secreted Cuvierian tubule adhesive (tubule prints) can be extracted using denaturing buffers containing both chaotropic and reducing agents. This soluble fraction contains about ten different proteins with molecular masses ranging from 10 to 220 kDa, but with closely related amino acid compositions, resembling that of the whole adhesive (De Moor et al. 2003). Non-secreted Cuvierian tubule adhesive has been directly extracted from isolated granular cells (adhesive cells). These cells were enzymatically dissociated from whole tubules and purified by density gradient centrifugation (Leclercq, Waite and Flammang, unpubl. data). Transmission electron microscopy demonstrated that granular cells were readily purified by this method (Fig. 10.8A). Extraction of the cells with the denaturing buffer used on tubule prints showed that their contents were much more easily solubilized than the secreted adhesive. Electrophoretic analyses revealed a very abundant low molecular weight protein (about 10 kDa) (Fig. 10.8B). The amino acid composition of this protein is almost identical to the one of the whole adhesive and to those of the proteins extracted from the tubule print material. The 10-kDa protein could therefore be the constitutive monomer of the adhesive. In this hypothesis, most of the proteins extracted from the secreted adhesive would be polymers of this 10-kDa protein. The chemical mechanism by which the adhesive monomer instantly polymerize upon release in sea water so far remains unknown.

Table 10.2. Amino acid compositions of adhesive secretions from the Cuvierian tubules of several species of holothuroids (values in residues per thousand)

Amino acid	*Holothuria forskali*[a]	*Holothuria leucospilota*[b]	*Bohadschia subrubra*[b]	*Pearsonothuria graeffei*[b]
HYP	0	24	8	8
ASX	78	74	64	62
THR	87	69	65	80
SER	60	42	58	58
GLX	91	122	106	124
PRO	55	74	69	63
GLY	266	267	298	254
ALA	88	115	91	85
CYS/2	14	3	9	4
VAL	38	29	35	37
MET	10	9	1	9
ILE	28	24	25	32
LEU	37	31	37	38
TYR	20	14	17	17
PHE	20	16	20	20
HIS	26	13	8	20
HLYS	0	5	12	3
LYS	31	12	29	22
ARG	50	57	46	63

[a] De Moor et al. (2003)
[b] Flammang et al. (2005)

10.5 Comparisons of Echinoderm Adhesives with Other Marine Bioadhesives

The density of seawater denies gravity the power to hold organisms to the bottom; thus, if they want to withstand the hydrodynamic forces, marine organisms must have adhesive mechanisms. Attachment to the substratum is therefore the most important use of adhesion by marine invertebrates, but other functions such as handling of food or building of tubes or burrows are also widespread in the marine fauna (Walker 1987; Tyler 1988; Flammang 1996; Whittington and Cribb 2001). Adhesion to the substratum may be permanent, transitory, or temporary (Tyler 1988; Flammang 1996; Whittington and Cribb 2001). Permanent adhesion involves the secretion of a cement and is characteristic of sessile organisms staying at the same place throughout their adult life (e.g., the attachment of barnacles on rocks). Transitory adhesion allows

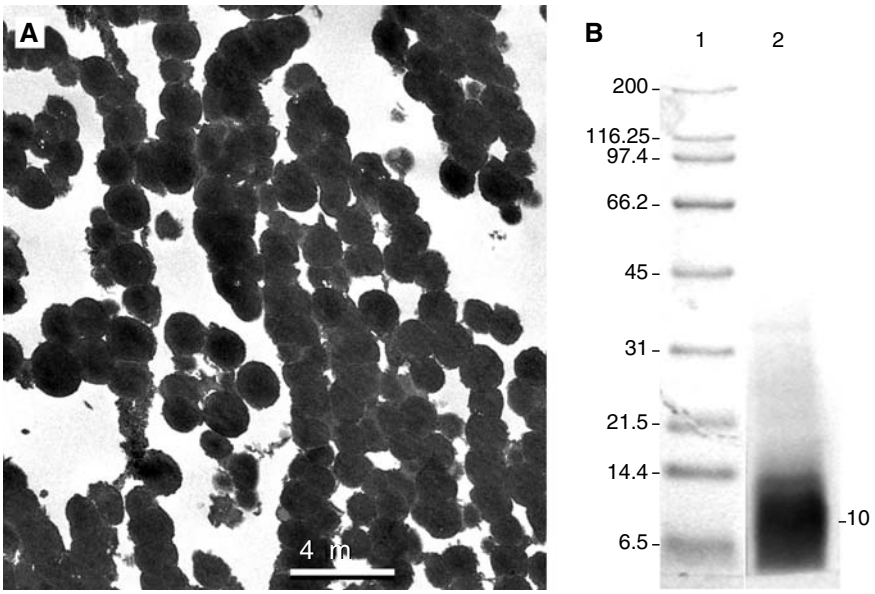

Fig. 10.8. Purification of the adhesive proteins from the Cuvierian tubules of *Holothuria forskali* (Leclercq, Waite and Flammang, unpubl. data): (a)transmission electron micrograph of a section through a pellet of purified granular cells; (b) electrophoresis (SDS-PAGE) of the proteins extracted from this pellet: *Lane 1*, molecular weight markers; *Lane 2*, extract from the granular cells showing a single abundant protein band at 10 kDa

simultaneous adhesion and locomotion: the animals attach by a viscous film they lay down between their body and the substratum, and creep on this film which they leave behind as they move (e.g., the ventral secretions of turbellarian platyhelminths). Temporary adhesion allows organisms to attach firmly but momentarily to a substratum (e.g., the adhesion of echinoderm podia). The boundary between transitory and temporary adhesion is not always clear, however. Indeed, gastropod molluscs may use either transitory adhesion (in conjunction with suction) when they are moving, or temporary adhesion when stationary for a long period of time, the latter giving by far the greatest adhesive strength to the animal (see, e.g., Smith et al. 1999a). A fourth type of adhesion, instantaneous adhesion, comprises invertebrate adhesive systems that do not fit into the three types of adhesion described above. These adhesive systems rely on single-use organs or cells and are used in functions other than attachment to the substratum requiring a very fast formation of adhesive bonds. Prey capture by collocyte-bearing tentacles of ctenophorans and defence reaction involving Cuvierian tubules in holothuroids are typical examples of this type of adhesion (Flammang et al. 2005).

In marine invertebrates, adhesive secretions are always predominantly made up of proteins. Yet their biochemical composition varies from one taxonomic group to another (Flammang et al. 1998a; Smith et al. 1999a;

Flammang 2003). As a general rule, permanent adhesives consist almost exclusively of proteins. On the other hand, non-permanent adhesives (transitory as well as temporary) are made up of an association of proteins and carbohydrates, the latter being mostly in the form of acid and sulfated sugars (see Whittington and Cribb 2001 for review). The ratio of proteins to carbohydrates is usually about 2:1 but there may be substantial variation on this figure though there is typically more protein than carbohydrate (Grenon and Walker 1980; Davies et al. 1990; Flammang et al. 1998a; Smith et al. 1999a; Smith and Morin 2002). The composition of the instantaneous adhesive of the Cuvierian tubule adhesive is reminiscent of non-permanent adhesives by its association of proteins and carbohydrate in a 3:2 ratio (De Moor et al. 2003). However, it differs from them by the fact that the carbohydrate fraction is in the form of neutral sugars and not acidic sugars.

As far as the amino acid composition of the protein fraction is concerned, all the marine bioadhesives characterized so far have in common their richness in small side-chain amino acids as well as in charged and polar amino acids. These characteristics were indeed observed in flatworms (Hamwood et al. 2002), mussels (Benedict and Waite 1986; Waite et al. 1989), limpets (Grenon and Walker 1980; Smith et al. 1999a), tubeworms (Jensen and Morse 1988; Stewart et al. 2004), barnacles (Walker 1972; Naldrett and Kaplan 1997; Kamino et al. 1996), sea stars (Flammang et al. 1998a), and sea cucumbers (De Moor et al. 2003). Charged and polar amino acids are probably involved in adhesive interactions with the substratum through hydrogen and ionic bonding (Waite 1987). Small side-chain amino acids, on the other hand, are often found in large quantities in elastomeric proteins (Tatham and Shewry 2000). These proteins are able to withstand significant deformations without rupture before returning to their original state when the stress is removed (Smith et al. 1999b). Marine glues thus appear to be tailored for both high adhesive strength and high cohesive strength.

Despite these similarities, the composition of marine invertebrate adhesives is variable from one species to another. To quantify this variability, the method of Marchalonis and Weltman (1971) was used. It allows the determination of relatedness among proteins based upon statistical analysis of differences in their amino acid composition. A parameter called $S\Delta Q$ is calculated by pairwise comparison of the percentages of each amino acid constituting the proteins. Marchalonis and Weltman (1971) reported that values of $S\Delta Q \leq 100$ indicate relatedness. Here, this method has been extended to whole adhesives, which are usually blends of different proteins, based on the assumption that if they include closely related proteins their whole amino acid compositions will be similar too. The values of $S\Delta Q$ for comparisons between the adhesives of 15 invertebrate species belonging to seven taxonomic groups are given in Table 10.3. Three amino acids (i.e., half-cystine, hydroxyproline and di-hydroxyphenylalanine [DOPA]) that were not considered by Marchalonis and Weltman (1971) have been taken into account because they are important constituents of some marine adhesives (Taylor

Table 10.3. SΔQ for comparison among adhesives in marine invertebrates (modified from Flammang 2003). Values in bold indicate relatedness; solid-line boxes frame species belonging to a same taxonomic group (i.e., mussels, barnacles, limpets and sea cucumbers, respectively)

		Permanent adhesion						Non-permanent adhesion				Instantaneous adhesion			
	Pc	Gd	Me	Bc	Be	Bh	Mr	Ll	Pv	Es	Ar	Hf	Hl	Bs	Pg
Pc	**0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gd	714	**0**	-	-	-	-	-	-	-	-	-	-	-	-	-
Me	706	**90**	**0**	-	-	-	-	-	-	-	-	-	-	-	-
Bc	989	191	348	**0**	-	-	-	-	-	-	-	-	-	-	-
Be	1038	263	430	**107**	**0**	-	-	-	-	-	-	-	-	-	-
Bh	888	216	383	**97**	**97**	**0**	-	-	-	-	-	-	-	-	-
Mr	907	160	364	**72**	**76**	**64**	**0**	-	-	-	-	-	-	-	-
Ll	1130	214	480	151	213	139	**77**	**0**	-	-	-	-	-	-	-
Pv	1093	176	385	**99**	128	**93**	**39**	**60**	**0**	-	-	-	-	-	-
Es	971	124	357	186	222	195	**78**	**105**	**96**	**0**	-	-	-	-	-
Ar	1007	116	323	**74**	148	**82**	**34**	**34**	**32**	**62**	**0**	-	-	-	-
Hf	818	145	131	433	580	457	427	445	406	345	345	**0**	-	-	-
Hl	1005	214	189	518	672	539	510	527	462	391	422	**40**	**0**	-	-
Bs	832	209	176	568	722	591	571	599	553	440	484	**28**	**34**	**0**	-
Pg	882	157	175	421	546	438	405	426	376	301	335	**20**	**23**	**34**	**0**

Tube-dwelling worm: Pc *Phragmatopoma californica*, Jensen and Morse 1988
Mussels: Gd *Geukensia demissa*, Waite et al. 1989; Me *Mytilus edulis*, Benedict and Waite 1986
Barnacles: Bc *Balanus crenatus*, Walker 1972; Be *Balanus eburneus*, Naldrett and Kaplan 1997; Bh *Balanus hameri*, Walker 1972; Mr *Megabalanus rosa*, Kamino et al. 1996
Limpets: Ll *Lottia limatula*, Smith et al. 1999; Pv *Patella vulgata*, Grenon and Walker 1980
Flatworm: Es *Entobdella soleae*, Hamwood et al. 2001
Sea star: Ar *Asterias rubens*, Flammang et al. 1998a
Sea cucumbers: Hf *Holothuria forskali*, De Moor et al. 2003; Hl *Holothuria leucospilota*, Bs *Bohadschia subrubra*, Pg *Pearsonothuria graeffei*, Flammang et al. 2005

and Waite 1997; Kamino et al. 2000). Aspartic acid and asparagine, glutamic acid and glutamine were taken as Asx and Glx respectively as in the original method. The level of significance was also set at 100, but two values just above 100 were also considered as indicating relatedness (Table 10.3). Values given in Table 10.3 show that the adhesives of every species within a same taxonomic group are related, suggesting, as expected, that they are homologous. More interesting is the relationship between the adhesives of all the species using non-permanent adhesion, despite the fact that they belong to very disparate phyla (i.e., platyhelminthes, mollusks and echinoderms; dotted-line box; Table 10.3). This relationship indicates convergence in composition because of common function and selective pressures. On the other hand, such an analogy is not observed for the adhesives of sessile invertebrates using permanent adhesion. Indeed, the adhesives from mussels, tubeworms and barnacles differ one from another (Table 10.3). The protein fractions of mussel byssal plaque and polychaete cement have in common the presence of DOPA in their composition (Benedict and Waite 1986; Jensen and Morse 1988; Waite et al. 1989). However, the tubeworm adhesive stands apart from any other adhesive by its very high content of phosphoserine (Stewart et al. 2004). Barnacle cement, on the other hand, contains no DOPA and appears to be closer to non-permanent adhesives (Table 10.3). They have in common the importance of disulfide bonds in their insolubilization (Flammang et al. 1998a; Smith et al. 1999a; Kamino et al. 2000; Hamwood et al. 2002). These disulfide bonds may be intermolecular, providing cross-linking between the adhesive proteins (Flammang et al. 1998a; Hamwood et al. 2002), or intramolecular, holding proteins in the specific shape required for interaction with their neighbors (Kamino 2001; Smith and Morin 2002). As for the instantaneous adhesive from holothuroid Cuvierian tubules, it differs from every other marine bioadhesive by its amino acid composition (Table 10.3). The protein fraction of this adhesive is particularly rich in glycine (De Moor et al. 2003; Flammang et al. 2005), resembling in this way mussel adhesives (Benedict and Waite 1986; Waite et al. 1989). To complete this comparison, marine invertebrate adhesives were plotted as a function of the hydrophobicity and polarity of their amino acids (Fig. 10.9) (Vincent 1980). Hydrophobicity was calculated using the method of Bigelow (1967). In this method, each amino acid that requires energy for transfer from a hydrophobic to an aqueous environment is assigned a value equal the energy of transfer minus the energy of transfer for glycine. The mole percent of each amino acid for which the free energy of transfer is positive is then multiplied by this free energy of transfer, and all the values are summed. Polarity is expressed as the percentage of polar amino acids in the composition. Once again, the non-permanent adhesives cluster together (except for the adhesive of *Patella vulgata* which has a lower percentage of polar amino acids) and are close to most barnacle adhesives (Fig. 10.9). With this representation, they are also close to mussel adhesives. The permanent adhesives from mussels, tubeworms, and barnacles largely differ one from another, and the instantaneous

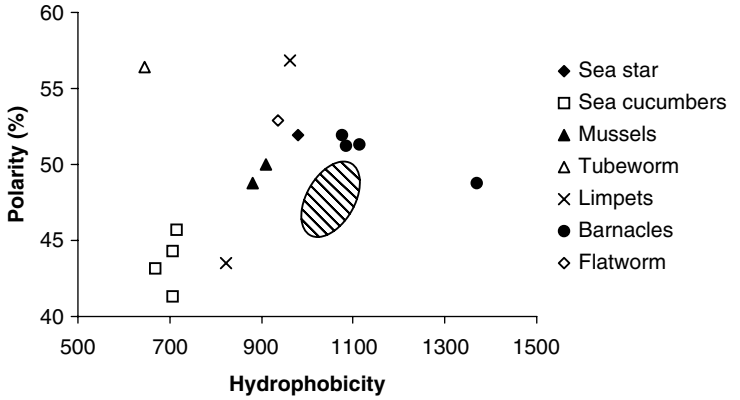

Fig. 10.9. Variation in hydrophobicity and polarity of amino acids in adhesives from marine invertebrates. The graph is based on the same amino acid compositions as those used in Table 10.3. The *striped area* represents average protein compositions compiled from different internet sources

adhesives from sea cucumbers stand apart from all other adhesives. Among marine bioadhesives, those of barnacles are the most hydrophobic and those of tubeworms and sea cucumber the least hydrophobic. Marine adhesives are blends of many different proteins and they are therefore compared to an average protein rather than to an arbitrarily selected protein (Fig. 10.9). As should be expected for an underwater glue, the different adhesives are either more polar (tubeworm, limpet, flatworm, sea star, barnacles) or more hydrophilic (sea cucumbers, limpets, mussels, flatworm, tubeworm) than the average protein composition, the two conditions not being mutually exclusive.

10.6 Conclusion

In addition to fundamental interests in marine bioadhesives, a substantial impetus behind understanding these adhesives are the potential technological applications that can be derived from their knowledge. These applications cover two broad fields of applied research: design of water-resistant adhesives and development of new antifouling strategies. The challenge with all of the biological adhesives is therefore to understand their mode of action sufficiently well that their essential features can be mimicked, in the case of the design of new adhesives, or inhibited, in the case of biofouling control. This will require a detailed knowledge of their physico-chemical characteristics (e.g., protein composition, sequences of these proteins, identification of their post-translational modifications), in order to relate them to their properties (e.g., specificity towards vari-

ous substrata, speed of action, insolubilization of the adhesive, life-expectancy of the attachment, etc.). This detailed information is progressively becoming available for the adhesive systems of several types of organisms (see the other chapters in this book). So far, however, none is available for any echinoderm adhesive secretion. Work is currently in progress to identify, purify, and characterize the different molecules involved in the reversible attachment of tube feet, the powerful fixation of asteroid larvae, and the instantaneous adhesion of Cuvierian tubules. Only the complete elucidation of their structure and characteristics will allow valid comparisons with the other biological adhesives.

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