

The glue of sea cucumber Cuvierian tubules: a novel marine bioadhesive

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Abstract

Several species of sea cucumbers possess a very peculiar and specialized defence system: the so-called Cuvierian tubules. It is mobilized when the animal is mechanically stimulated, resulting in the discharge of a few white filaments, the tubules. In seawater, the expelled tubules considerably lengthen and become sticky upon contact to any object. The great adhesivity of the outer epithelium combined with the high tenacity of the collagenous core makes Cuvierian tubules very efficient at entangling and immobilizing most potential predators. The fact that Cuvierian tubules are able to form strong adhesive bonds in a few seconds to a variety of substrata suggests that their glue could offer novel features or performance characteristics for applications as underwater adhesives. This paper compares some recent data on the mechanical and biochemical properties of the adhesive from holothuroid Cuvierian tubules with the properties of the adhesives from other marine invertebrates. The adhesive of Cuvierian tubules stands apart from every other marine invertebrate adhesive described so far by its gross biochemical composition as well as by the amino acid composition of its protein fraction. Yet, the adhesive strength of this organ falls within the range of tenacities measured for other adhesive systems. Cuvierian tubules could therefore be an interesting model system in the search for a novel bioadhesive with biotechnological applications.

Introduction

Adhesion (attachment with adhesive substances) is a way of life in the sea. Indeed, representatives of bacteria, protocists (including macroalgae), and all animal phyla living in the sea attach to surfaces, including other organisms (Walker, 1987). Adhesion is particularly developed and diversified in invertebrates. It is involved in various functions such as the attachment of the larvae and/or the adults to the substratum, the locomotion, the taking of food, or the building of tubes or burrows (Walker, 1987; Tyler, 1988; Flammang, 1996). Adhesion may be permanent as in sessile invertebrates that cement themselves to the substratum (e.g., barnacles), or non-permanent as in those benthic organisms that

move around at some times and attach themselves strongly but temporarily to the substratum at other times (e.g., limpets) (Walker, 1987; Tyler, 1988; Flammang, 1996).

The fact that marine invertebrates produce adhesives that act in the presence of water has aroused increasing scientific and technological attention because such adhesives are sorely needed for applications in underwater construction or in the medical and dental fields (Strausberg & Link, 1990; Yamamoto, 1995; Taylor & Waite, 1997). Most studies of invertebrate adhesive systems have focused on the characterization of the permanent adhesives from sessile organisms such as mussels, barnacles or tube-dwelling worms (see, e.g., Naldrett & Kaplan, 1997; Taylor & Waite, 1997; Kamino *et al.*, 2000). This is because in these organisms the adhesives are secreted as a fluid and then gradually solidify to form a cement possessing high adhesive and cohesive strengths (Walker, 1987). However, the adhesive biochemistry of sessile invertebrates has proven to be quite complex: not only do the adhesives consist of a blend of several different proteins (Taylor & Waite, 1997; Kamino *et al.*, 2000; Waite & Qin, 2001) but their hardening relies on enzymatic modifications of the secreted proteins (Dougherty, 1997; Hansen *et al.*, 1998). So far, non-permanent adhesives have attracted much less interest and the best characterized is that from the limpets. In these organisms, the adhesive is a hydrogel comprising high molecular weight polysaccharides and low molecular weight proteins associated in a non-covalently linked complex (Grenon & Walker, 1980; Smith *et al.*, 1999a).

In the search for simpler models of bioadhesion, other adhesive systems from marine invertebrates are therefore also worth investigating. One of these systems is the defensive reaction occurring in some species of holothuroid echinoderms (sea cucumbers), and which involves the Cuvierian tubules (Flammang, 1996; Hamel & Mercier 2000). Several species of holothuroids, all belonging exclusively to the family Holothuriidae, possess this very peculiar and specialized defence system. It is mobilized when the animal is mechanically stimulated, resulting in the discharge of a few white filaments, the tubules. In seawater, the expelled tubules lengthen considerably and become sticky upon contact with any object (VandenSpiegel & Jangoux, 1987). The great adhesivity of the outer tubule epithelium combined with the high tensile strength of their collagenous core make Cuvierian tubules very efficient for entangling and immobilizing most potential predators (VandenSpiegel & Jangoux, 1987; Hamel & Mercier, 2000).

This paper compares some recent data on the mechanical and biochemical properties of the adhesive from holothuroid Cuvierian tubules with the properties of the adhesives from other marine invertebrates. Its aim is to find out if Cuvierian tubule glue could offer novel features or performance characteristics for applications as an underwater adhesive.

Adhesive strength

The evaluation of the adhesive strength in marine invertebrates is usually done by measuring their tenacity, which is the adhesion force per unit area and is expressed in Pascals (Pa). According to the taxonomic group considered, tenacities of marine organisms range from about 1 to 2000 kPa (see, e.g., Walker, 1987, for review). However, many studies have shown that several factors may profoundly influence the tenacity of invertebrates (see, e.g., Grenon & Walker, 1981). For example, the physical (e.g., roughness) as well as chemical characteristics (e.g., hydrophobicity, surface charges) of the substratum are known to change the tenacity of organisms by up to an order of magnitude (Young & Crisp, 1982; Yule & Walker, 1987). Adhesion force measurements can also be performed using either a pull at right angle to the substratum (normal), a pull parallel to the substratum (shear), or a combination of both. It was demonstrated that the direction of pull also strongly influences the measured tenacity of marine invertebrates (Grenon & Walker, 1981). As a consequence, great care should be exercised when comparing values of tenacity extracted from different studies. Table 1 presents the tenacities of various marine invertebrates, all measured on smooth glass and by pulling the organism or its adhesive organ normal to the substratum. Comparison of the values indicates that organisms attaching permanently show a higher tenacity than organisms attaching temporarily.

Cuvierian tubule adhesive strength on glass has been measured in seven species of sea cucumbers belonging to the genera *Bobadschia*, *Holothuria* and *Pearsonothuria* (Flammang *et al.*, unpubl. obs.). The mean normal tenacities observed varied from about 30 to 135 kPa. These tenacities fall within the range of adhesive strengths described for marine organisms (tab. 1). They lie, however, among the lowest values observed, being closer to the tenacity of organisms using non-permanent adhesion than to those of sessile marine invertebrates using permanent adhesion. In natural conditions, however, Cuvierian tubule adhesion functions under shear loading and not under orthogonal loading. The shear tenacity of Cuvierian tubules can be at least four times higher than their normal tenacity. For example, the normal tenacity of the tubules of *Holothuria forskali* on paraffin wax is about 4 kPa (Flammang *et al.*, unpubl. obs.) whereas shear tenacity on the same substratum is about 15 kPa (Zahn *et al.*, 1973). This is because in the case of a cylindrical structure like a tubule, a pull normal to the substratum induces extensive peeling during detachment. On the other hand, peeling does not occur when Cuvierian tubules are detached in shear (Zahn *et al.*, 1973). It is well-known that peeling dramatically reduces adhesion in marine organisms. For example, in the sea anemone *Actinia equina*, Young *et al.* (1988) increased the measured normal adhesive strength on Tufnol (a plastic) from 20 kPa to 460 kPa by just switching to an experimental design that reduced the incidence of peel. This means that the

tenacities of sea cucumber Cuvierian tubules presented in table 1 are probably underestimates and suggests that Cuvierian tubules compare well with adhesive systems of other marine invertebrates in terms of adhesive strength.

Table 1 - Comparison of the mean normal tenacity of some marine invertebrates on smooth glass. When two values are indicated, they represent minimum and maximum means from different species, except in *Mytilus edulis* for which they represent measurements made at different seasons.

Taxa	Species	Structure involved in adhesion	Tenacity (kPa)	Reference
Permanent adhesion				
Mollusca Bivalvia	<i>Mytilus edulis</i> (February-June)	Byssus	316-750	Young & Crisp, 1982
Crustacea Cirripedia (adult)	<i>Balanus crenatus</i> , <i>Semibalanus balanoides</i>	Base	230-520	Yule & Walker, 1987
Non-permanent adhesion				
Cnidaria Anthozoa	<i>Actinia equina</i> , <i>Metridium senile</i>	Foot	19-43	Young <i>et al.</i> , 1988
Mollusca Gasteropoda	<i>Patella vulgata</i>	Foot	228	Grenon & Walker, 1981
Crustacea Cirripedia (cyprid larva)	<i>Semibalanus balanoides</i>	Antennules	98	Yule & Walker, 1987
Echinodermata Asteroidea	<i>Asterias rubens</i> , <i>Asterias vulgaris</i>	Tube feet	170-198	Paine, 1926; Flammang & Walker, 1997
Cuvierian tubule adhesion				
Echinodermata Holothuroidea	<i>Bobadschia marmorata</i> , <i>Bobadschia subrubra</i> , <i>Holothuria forskali</i> , <i>Holothuria impatiens</i> , <i>Holothuria leucospilota</i> , <i>Holothuria maculosa</i> , <i>Pearsonothuria graeffei</i>	Cuvierian tubules	30-134	Flammang <i>et al.</i> , unpubl. obs.

Composition of the adhesive

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.*, 1998; Smith *et al.*, 1999a). As a general rule, permanent adhesives consist almost exclusively of proteins: for example, mussel byssal plaques contain about 99% proteins (Cook, 1970) and barnacle cement about 85% (Walker, 1972). On the other hand, non-permanent adhesives are made up of an association of proteins and carbohydrates, usually in a 2:1 ratio and that together represents about 50% of the adhesive dry weight (see Whittington & Cribb, 2001, for review). The carbohydrate fraction is mostly in the form of acidic and sulphated sugars and associated to an important inorganic fraction accounting for the other 50% of the adhesive material (Grenon & Walker, 1980; Flammang *et al.*, 1998; Smith

et al., 1999a). The Cuvierian tubule adhesive is made up essentially of organic material (about 90%), the inorganic residue amounting only to about 10% (De Moor *et al.*, 2002). The organic fraction comprises both proteins and carbohydrates in a 3:2 ratio. The composition of the Cuvierian tubule adhesive is therefore reminiscent of non-permanent adhesives by its association of proteins and carbohydrates. However, it differs from them by the fact that the carbohydrate fraction is in the form of neutral sugars and not acidic sugars, and by the fact that the inorganic fraction is much smaller. Cuvierian tubule adhesive thus appears as unique among invertebrate adhesives in terms of its gross biochemical composition.

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 & Waite, 1986; Waite *et al.*, 1989), limpets (Grenon & Walker, 1980; Smith *et al.*, 1999a), tubeworms (Jensen & Morse, 1988), barnacles (Walker, 1972; Kamino *et al.*, 1996; Naldrett & Kaplan, 1997), sea stars (Flammang *et al.*, 1998), and sea cucumbers (De Moor *et al.*, 2002). 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 & 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 SAQ is calculated by pairwise comparison of the percentages of each amino acid constituting the proteins. Marchalonis and Weltman (1971) reported that values of SAQ ≤ 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 enclose closely related proteins their whole amino acid compositions will be similar too. The values of SAQ for comparisons between the adhesives of twelve invertebrate species belonging to seven taxonomic groups are given in table 2. Three amino acids (i.e., half-cystine, hydroxyproline and di-hydroxyphenylalanine [DOPA]) that were not considered by Marchalonis & Weltman (1971) have been taken into account because they are important constituents of some marine adhesives (Taylor & Waite, 1997; Kamino *et al.*, 2000). Aspartic acid and asparagine, glu-

tamic 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 than 100 were also considered as indicating relatedness (tab. 2). Values given in table 2 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, molluscs and echinoderms; table 2). 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 (tab. 2). The protein fractions of mussel byssal plaque and polychaete cement have in common the presence of DOPA in their composition (Benedict & Waite, 1986; Jensen & Morse, 1988; Waite *et al.*, 1989). This unusual amino acid is involved in surface coupling (adhesion) and cross-linking (cohesion) of these adhesives (Taylor & Waite, 1997). However, the tubeworm adhesive stands apart from any other adhesive by its very high content of serine (Jensen & Morse, 1988). Barnacle cement, on the other hand, contains no DOPA and appears to be closer to non-permanent adhesives (tab. 2). They have in common the importance of disulphide bonds in their cross-linking (Flammang *et al.*, 1998; Smith *et al.*, 1999a; Kamino *et al.*, 2000; De Moor *et al.*, 2002). It was already suggested that barnacle adhesive would function as a highly viscous fluid and not as a true solid cement (see Yule & Walker, 1987, for review). This hypothesis was based on the fact that juvenile barnacles can move at least 2 cm under the pressure of neighbours. It is further corroborated by the similarity of the composition of the protein fraction from barnacle adhesive with that of viscous non-permanent adhesives. As for the adhesive from holothuroid Cuvierian tubules, it differs from every other marine bioadhesive by its amino acid composition (tab. 2). The protein fraction of this adhesive is particularly rich in glycine (De Moor *et al.*, 2002), resembling in this way mussel adhesives (Benedict & Waite, 1986; Waite *et al.*, 1989).

Table 2 - Values of SAQ for comparison among adhesives in marine invertebrates. Values in bold indicate relatedness; boxes frame species belonging to a same taxonomic group (i.e., mussels, barnacles and limpets, respectively). CT, Cuvierian tubule adhesion.

	Permanent adhesion							Non-permanent adhesion				CT
	Gd	Me	Pc	Bc	Be	Bh	Mr	Es	Ll	Pv	Ar	Hf
Gd	0	-	-	-	-	-	-	-	-	-	-	-
Me	90	0	-	-	-	-	-	-	-	-	-	-
Pc	714	706	0	-	-	-	-	-	-	-	-	-
Bc	191	348	989	0	-	-	-	-	-	-	-	-
Be	263	430	1038	107	0	-	-	-	-	-	-	-
Bh	216	383	888	97	97	0	-	-	-	-	-	-
Mr	160	364	907	72	76	64	0	-	-	-	-	-
Es	124	357	971	186	222	195	78	0	-	-	-	-
Ll	214	480	1130	151	213	139	77	105	0	-	-	-
Pv	176	385	1093	99	128	93	39	96	60	0	-	-
Ar	116	323	1007	74	148	82	34	62	34	32	0	-
Hf	145	131	818	433	580	457	427	345	445	406	345	0

Mussels: Gd, *Geukensia demissa*, Waite et al., 1989; Me, *Mytilus edulis*, Benedict & Waite, 1986. Tube-dwelling worm: Pc, *Phragmatopoma californica*, Jensen & Morse, 1988. Barnacles: Bc, *Balanus crenatus*, Walker, 1972; Be, *Balanus eburneus*, Naldrett & Kaplan, 1997; Bh, *Balanus hameri*, Walker, 1972; Mr, *Megabalanus rosa*, Kamino et al., 1996. Flatworm: Es, *Entobdella soleae*, Hamwood et al., 2002. Limpets: Ll, *Lottia limatula*, Smith et al., 1999a; Pv, *Patella vulgata*, Grenon & Walker, 1980. Sea star: Ar, *Asterias rubens*, Flammang et al., 1998. Sea cucumber: Hf, *Holothuria forskali*, De Moor et al., 2002.

Conclusion and prospects

The best-characterized marine bioadhesive is that from the blue mussel, *Mytilus edulis*. In this species, several proteins have been identified and characterized that co-occur as a complex blend in the byssal adhesion plaques (Waite, 1992; Taylor & Waite, 1997; Waite & Qin, 2001). So far, however, only one of these proteins (Mefp-1) has been used in biotechnological applications. Several imaginative applications have been developed for this protein (or for derived peptides), including, among others: tissue adhesives as sealants for medical, surgical and dental applications; enzyme, cell, and tissue-immobilizing agents; anti-corrosives and metal scavengers (Burzio et al., 1997; Taylor & Waite, 1997). However, several limitations have come out such as the requirement of post-translational modifications to certain amino acids, or the need for a separate enzyme for curing (Strausberg & Link, 1990). New models of bioadhesion are therefore sought that could overcome these problems.

The adhesive of sea cucumber Cuvierian tubules stands apart from every other marine invertebrate adhesive described so far by its gross biochemical composition as well as by the amino acid composition of its protein fraction. Yet, the adhesive strength of this organ falls within the range of tenacities measured for other adhesive systems. Moreover, it possesses some interesting characteristics like its capacity to form adhesive bonds in a matter of seconds (less than 10 s; Zahn et al., 1973).

Cuvierian tubule adhesive could therefore offer novel features or performance characteristics for biotechnological applications. Work is currently in progress to identify, purify and characterize the constitutive proteins of Cuvierian tubule adhesive. The complete elucidation of their structure and physico-chemical characteristics is an obligatory prerequisite before any application can be envisaged.

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