

PROTEIN PHOSPHORYLATION: A WIDESPREAD MODIFICATION IN MARINE ADHESIVES

Patrick Flammang, Aurélie Lambert, Emeline Wattier & Elise Hennebert
University of Mons-Hainaut, Marine Biology Laboratory, B-7000 Mons, Belgium
Patrick.Flammang@umh.ac.be

Introduction

In the marine environment, attachment mechanisms developed by animals usually rely on highly viscous or solid adhesive secretions (1). These secretions always contain specialized proteins: the marine adhesive proteins. Functional convergences are noted among marine animals, particularly in terms of the type of adhesion used, permanent, temporary or instantaneous (2). Permanent adhesion is characteristic of sessile organisms that cement themselves to the substratum and in which adhesives are secreted as a fluid which then gradually solidifies to form a cement (*e.g.*, mussels, barnacles and tube-dwelling worms). Temporary adhesion occurs in those benthic animals that attach themselves strongly but temporarily to the substratum through visco-elastic secretions, and therefore keep the capacity to move around (*e.g.*, limpets, sea stars). Instantaneous adhesion comprises invertebrate adhesive systems relying on single-use organs or cells and used in prey capture or defense (*e.g.*, ctenophore [comb jellies] tentacles, sea cucumber Cuvierian tubules). Although little information is available about marine adhesive proteins, molecular convergences have also been pointed out. Indeed, proteins from animals as different as mollusks, annelids or tunicates all enclose the unusual amino acid 3,4-dihydroxy-L-phenylalanine (DOPA), which is involved in surface coupling and solidification of the adhesive (3). There are, however, many marine adhesive proteins that do not rely on DOPA for their function, and other shared adhesive motifs probably exist. Recently, another modified amino acid, phosphoserine (pSer), has emerged as an important motif in biological adhesives (4). During the last decade, Herbert Waite and his group have characterized several polyphosphoproteins (*i.e.* proteins containing numerous pSer residues) from the adhesive secretions of two marine organisms, mussels and tube-building worms of the family Sabellariidae (5-7). pSer is essentially a monophosphoester negatively charged at the pH of seawater, rendering this residue and the sequence surrounding it highly polar (4). In the adhesive secretions of marine invertebrates, phosphorylation is thought to impart a potential for (a) protein condensation in secretory granules through a process called complex coacervation (6,7); (b) cohesive (by Ca^{++} bridging) and adhesive contributions to the glue (7,8); and (c) protein-protein cross-linking through histidinoalanine crosslinks (9). The occurrence of polyphosphoproteins in two unrelated adhesive systems (mussel and tube-building worm) suggests the possibility that such proteins could be important components also in other broad-

hesives, and may therefore present a wider distribution within the animal kingdom.

Experimental

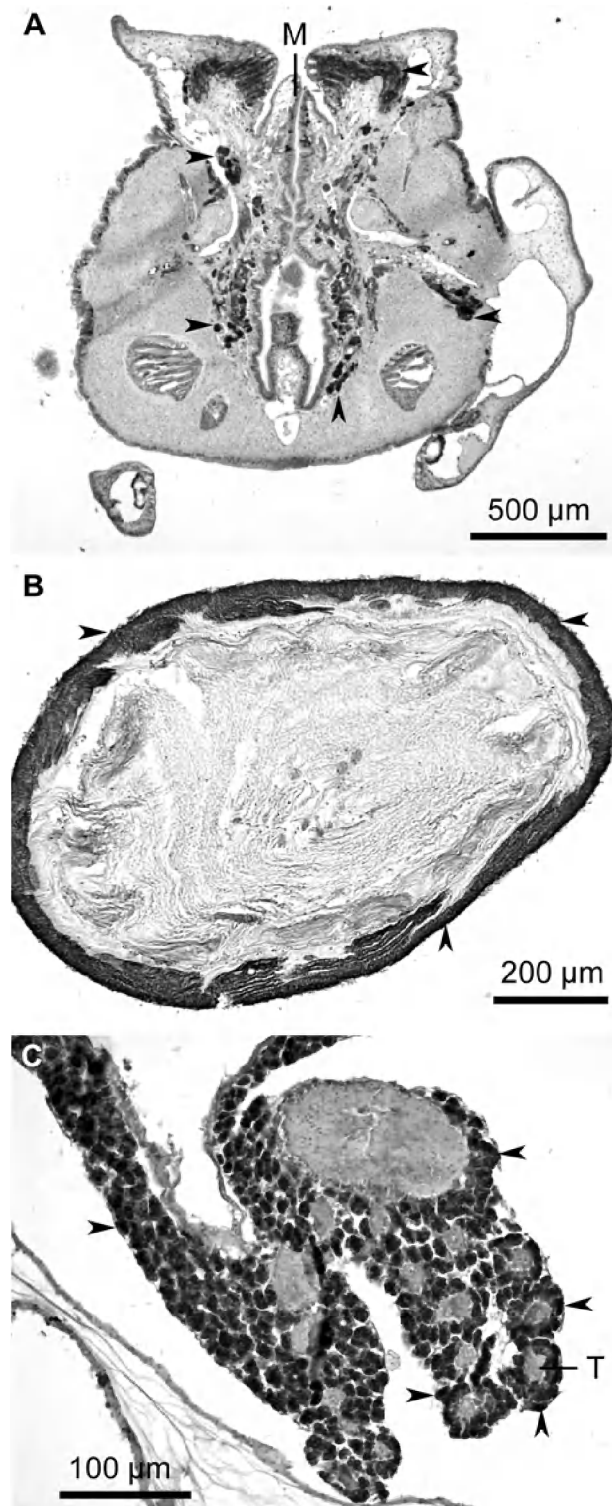
Adhesives organs from different marine invertebrates were fixed in Bouin's fluid, embedded using a routine method in paraffin wax, and sectioned at 7 μm . Sections were then subjected to an indirect immunohistochemical staining method using monoclonal anti-phosphoserine antibodies (clone PSR-45, mouse ascites fluid; Sigma, P 3430) and observed with a Zeiss Axioscope A1 microscope.

Results and Discussion

To investigate whether antibodies directed against pSer could be used to specifically label polyphosphoproteins in marine adhesives, they were applied on paraffin sections through the adhesive organs of the mussel *Mytilus edulis* and of the sabellariid polychaete *Sabellaria alveolata*. In both cases, a strong anti-pSer labeling was detected in the adhesive glands (phenol gland and cement gland [Fig. 1A], respectively). Most labeling was suppressed by pre-incubation of the antibodies with casein, a milk polyphosphoprotein. Although mussel and tube-worm adhesive proteins have in common the presence of many phosphorylated serine residues in their sequence, they appear to be unrelated phylogenetically (4). Yet, this shared post-translational modification allows the use of a single anti-pSer monoclonal antibody to specifically label these otherwise non-homologous proteins. Anti-pSer antibodies therefore appear as a powerful tool to specifically label adhesive polyphosphoproteins.

The antibodies were then used on sections from adhesive organs of three other organisms: the tube feet of the sea star *Asterias rubens*, the Cuvierian tubules of the sea cucumber *Holothuria forskali*, and the tentacles of the comb jelly *Pleurobrachia pileus*. Sea star tube feet rely on temporary adhesion for their operation while sea cucumber Cuvierian tubules and ctenophore tentacles are typical examples of instantaneous adhesion (2). Although sea stars showed no anti-pSer immunoreactivity, there was an extensive immunolabeling of the adhesive cells of sea cucumbers and ctenophores. In Cuvierian tubules, the thick outer adhesive epithelium (*i.e.* the mesothelium; 2) is strongly labelled at the level of its constituting granular cells (Fig. 1B). Ctenophore tentacles bear numerous projections, the tentillae, covered externally by specialized adhesive cells, the so-called colocytes (10). These collo-

cytes are extensively labelled by the anti-pSer antibodies (Fig. 1C).



The occurrence of polyphosphoproteins in the adhe-

sives of sea cucumber Cuvierian tubules and ctenophore tentacles indicates that they could share some molecular mechanisms with the adhesives of mussels and sabellariid tube-worms, even though they represent different types of adhesion. Indeed, mussel byssal plaques and sabellariid cement are both permanent adhesives which are initially secreted as fluids and then gradually solidify to form glues possessing high adhesive and cohesive strength (2,3). Curing involves another modified amino acid that mussel and polychaete adhesives have in common in their adhesive proteins, DOPA (7). Sea cucumber Cuvierian tubules and ctenophore tentacles, on the other hand, are a typical examples of instantaneous adhesion, a type of adhesion used in functions requiring a very fast formation of adhesive bonds such as defense reactions and prey capture (2). Instantaneous adhesives are not designed to last and do not appear to be cured (11,12). Yet, many of the functions proposed for polyphosphoproteins in the adhesives of mussels and tube-worms (condensation, adhesion and cohesion) may apply to their equivalents in instantaneous adhesives as well.

Our findings bring to four the number of polyphosphoprotein-containing marine adhesives and raise questions about the convergent evolution of these adhesives (Fig. 2). Interestingly, in these four marine organisms, the secretory granules of the adhesive cells are strikingly similar. In light microscopy, they stain with acidic dyes such as eosin or azocarmine (10,13-15) while in TEM they reveal a homogeneous electron-dense content (2,10,15,16). Adhesive granules with an acidophilic and osmiophilic content are relatively common in the animal kingdom (17), suggesting that polyphosphoprotein-containing marine adhesives might be even more widely distributed (Fig. 2). Similar adhesive granules have been observed for example in flatworms (18), in cephalopods (19), as well as in the larvae of many marine benthic organisms such as bryozoans, cirripede crustaceans and tunicates (20). The comparative immunohistochemical approach using anti-pSer antibodies used in this study is currently continuing to assess the distribution of polyphosphorylated proteins in the adhesive organs of marine invertebrates.

←

Figure 1. Marine invertebrate adhesive organs immunolabeled with anti-pSer antibodies (the immunopositive cells, appearing in black, are indicated by arrowheads). (A) Transverse section through the tube-worm *Sabellaria alveolata* at the level of the building organ (the mouth [M] faces upwards). (B) Transverse section through a Cuvierian tubule of the sea cucumber *Holothuria forskali*. (C) Transverse section through a retracted tentacle of the ctenophoran *Pleurobrachia pileus* showing several tentillae [T] in cross-section.

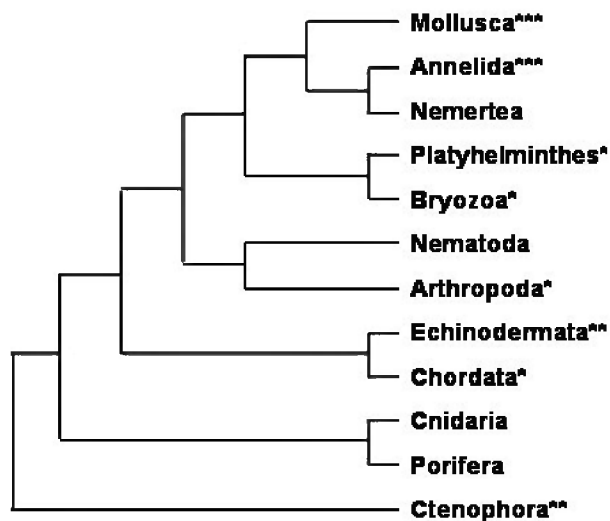


Figure 2. The phylogeny of polyphosphoprotein-containing adhesives in the animal kingdom. Phyla with asterisks are those with species using or presumably using such adhesives, evidence for pSer being derived from biochemical analyses (***), immunolabeling (**) or tinctorial affinities (*) of the adhesive organs. Phylogenetic tree is adapted from (21)

Acknowledgements

Our warmest thanks to Prof. Herbert Waite who inspired us this study. E.H. benefited from a FRiA doctoral grant (Belgium). P.F. is Senior Research Associate of the Fund for Scientific Research of Belgium (F.R.S.-FNRS). Work supported by a F.R.S.-FNRS Grant n° F.4501.07. This study is a contribution of the “Centre Interuniversitaire de Biologie Marine” (CIBIM).

References

1. A.M. Smith and J.A. Callow, *Biological Adhesives*, 2006, Springer Verlag, Berlin.
2. P. Flammang, *Biological Adhesives* (2006, Springer Verlag, Berlin). Chap. 10, pp. 183-206.
3. Taylor, S.W., Waite, J.H., *Protein-Based Materials* (1997, Birkhäuser, Boston). Chap. 7, pp. 217-248.
4. J. Sagert, C. Sun and J.H. Waite, *Biological Adhesives* (2006, Springer Verlag, Berlin). Chap. 7, pp. 125-143.
5. J.H. Waite and X. Qin, *Biochemistry* **40**, 2001, pp 2887-2893.
6. R.J. Stewart, J.C. Weaver, D.E. Morse and J.H. Waite, *J. Exp. Biol.* **207**, 2004, pp 4727-4734.
7. H. Zhao, C. Sun, R.J. Stewart and J.H. Waite, *J. Biol. Chem.* **280**, 2005, pp 42938-42944.
8. C. Sun, G.E. Fantner, J. Adams, P.K. Hansma and J.H. Waite, *J. Exp. Biol.* **210**, 2007, pp 1481-1488.
9. C.M. Taylor and W. Wang, *Tetrahedron Rep.* **63**, 2007, pp 9033-9047.
10. J.M. Franc, *Biol. Bull.* **155**, 1978, pp 527-541.
11. P. Flammang, J. Ribesse and M. Jangoux, *Integr. Comp. Biol.* **42**, 2002, pp 1107-1115.
12. W.E.G. Müller and R.K. Zahn, *Cytobiologie* **5**, 1972, pp 335-351.
13. J. Vovelle, *Arch. Zool. Exp. Gén.* **106**, 1965, pp 1-187.
14. C.H. Brown, *Q. J. Microsc. Sci.* **93**, 1952, pp 487-503.
15. D. VandenSpiegel and M. Jangoux, *Mar. Biol.* **96**, 1987, pp 263-275.
16. A. Tamarin and P.J. Keller, *J. Ultrastruct. Res.* **40**, 1972, pp 401-416.
17. S. Tyler, *Fortsch. Zool.* **36**, 1988, pp 331-347.
18. I.D. Whittington and B.W. Cribb, *Adv. Parasitol.* **48**, 2001, pp 101-224.
19. J. von Byern and W. Klepal, *Biofouling* **22**, 2006, pp 329-338.
20. D.J. Crisp, *Marine Biodeterioration: An Interdisciplinary Study* (1984, Spon, London), pp 103-126.
21. C.W. Dunn et al., *Nature* **452**, 2008, pp 745-749.