

The tube cement of *Phragmatopoma californica*: a solid foam

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Summary

Phragmatopoma californica is a marine polychaete that builds protective tubes by joining bits of shell and sand grains with a secreted proteinaceous cement. The cement forms a solid foam (closed cells) via covalent crosslinking, as revealed by electron and laser scanning confocal microscopy. The cement contains extractable calcium and magnesium, and non-extractable phosphorus. Amino acid analysis demonstrated that the phosphorus is in the form of phosphoserine and that >90% of serine in the cement (i.e. 28 mol% of residues) is phosphorylated. In addition to previously identified basic proteins, the cement contains a highly acidic polyphosphoserine protein as a major component. We propose a model for the structure and bonding mechanism of the cement that has the following major features: (1) within the secretory pathway of cement gland cells, the electrostatic association of the

oppositely charged proteins and divalent cations (Ca²⁺ and Mg²⁺) condense the cement proteins into dehydrated secretory granules; (2) the condensation of the cement leads to the separation of the solution into two aqueous phases (complex coacervation) that creates the closed cell foam structure of the cement; (3) rehydration of the condensed cement granules after deposition onto tube particles contributes to the displacement of water from the mineral substrate to facilitate underwater adhesion; and (4) after secretion, covalent cross-linking through oxidative coupling of DOPA gradually solidifies the continuous phase of the cement to set the porous structure.

Key words: polychaeta, sabelliidae, *Phragmatopoma californica*, bioadhesion, complex coacervation, polyphosphoserine.

Introduction

The sabelliids are gregarious obligate tube-dwelling marine polychaetes (Ruppert and Barnes, 1994). The sabelliids have an unusual strategy for constructing their mineralized tubes. Rather than synthesizing a complete mineralized structure by the controlled precipitation of concentrated ions with matrix proteins, like many other tube- or shell-dwelling marine invertebrates (Simkiss, 1986), the sabelliids gather the mineral phase adventitiously as preformed particulates from the water column, usually sand and bits of calcareous shell of the right size, and secrete only a proteinaceous cement for joining the particles. Captured particles are conveyed along the tentacles of the crowns to the building organ, a U-shaped invagination near the mouth, where they are held, turned and evaluated for size, shape and composition. Particles found to be satisfactory are judiciously dabbed with spots of cement secreted from thoracic cement glands, then pressed into place at the end of the tube by the building organ, in a manner reminiscent of stonemasonry (Eckelbarger, 1978).

The sabelliids are commonly called sandcastle worms because individual tubes with their resident worm are honey-combed together into large reef-like mounds, although the

mounds more-closely resemble proletarian apartment buildings than castles. Their gregariousness is due to a component of the tube cement that induces larvae to settle, metamorphose and build a new tube on existing conspecific tubes (Eckelbarger, 1978; Jensen, 1992). The colonies occur in the inter-tidal zone where there is sufficient wave action to suspend food and appropriate particles for tube building and repair. The sandcastle construction, and in particular the cement bonds, must therefore be robust enough to withstand the siege of a turbulent, high-energy environment. The cement is an important model for biomimetic adhesives because of its apparent toughness, because it adheres strongly to a variety of materials, and because it bonds rapidly to these materials in seawater.

Two cement precursor proteins, Pc1 and Pc2, have been isolated from the cement glands of *Phragmatopoma californica* (Waite et al., 1992), a sabelliid that lives off the coast of California. Both precursor proteins are basic (predicted pI values of 9.7 and 9.95, respectively, from unpublished gene sequences) and consist of repeated sequence motifs rich in glycine, lysine, and 3,4-dihydroxyphenyl-L-alanine (DOPA) residues. The DOPA functional groups may

participate in bonding of the cement to mineral surfaces as well as quinone-tanning of the cement during hardening (Waite, 1999). In addition to the DOPA-containing Pc1 and Pc2, at least one more protein rich in serine was suggested by amino acid analysis of the whole cement. Ser content of whole cement approaches 28 mol%, whereas isolated Pc1 and Pc2 contained only 3.7 and 2.5 mol% serine, respectively (Jensen and Morse, 1988; Waite et al., 1992). The tube cements of other sabellariid polychaetes have been shown by elemental analysis to contain inorganic elements, namely phosphorus, magnesium and calcium in relatively large amounts, and in some cases perhaps traces of manganese, iron, zinc and aluminum (Gruet et al., 1987; Truchet and Vovelle, 1977b; Vovelle, 1979). In all cases, the phosphorus, calcium and magnesium were perfectly co-localized both in the external cement and in the secretory granules of the cement glands. Here, we report further characterization of the structure and composition of the cement of *P. californica*, and present a model that accounts for the cement structure and mechanism of bonding.

Materials and methods

Harvesting P. californica cement

Colonies of *Phragmatopoma californica* Fewkes were collected at sites near Goleta, CA, during January 2004 and maintained in the laboratory for several months in flow tanks flushed with filtered natural seawater at 7°C. To collect tube cement, individual worms were removed from the colony with about 1 cm of their tubes intact and placed on a bed of 0.5 mm glass beads or other particles. Previous studies had shown that the preferred diameter of glass beads was 0.5 mm (Jensen and Morse, 1988). Most of the animals promptly extend their tubes with the glass beads. Sections of the rebuilt tube were removed from the tubes with forceps without harming the animal, washed extensively with de-ionized water, then processed immediately for analysis or frozen in de-ionized water and stored at -80°C.

Scanning electron microscopy and energy dispersive spectrometry

For electron microscopy, glass beads containing fresh glue disks either were mounted on conductive carbon tabs (Ted Pella; Redding, CA, USA) on SEM posts as individual beads and lyophilized, or mounted as intact sections of glass tubes, then lyophilized. In the latter case, beads were broken apart after lyophilization. The samples were sputter-coated with gold using a Denton Vacuum DESK II coater (Moorestown, NJ, USA), and examined with a Tescan Vega TS 5130MM thermionic emission scanning electron microscope equipped with an IXRF Systems energy dispersive spectrometer (Houston, TX, USA).

Laser scanning confocal microscopy

Intact cement disks were pried off glass beads with a forceps and mounted in seawater between a coverslip and microscope slide then sealed with fingernail polish. The samples were

examined under a 60× planapochromat objective, numerical aperture 1.4, with an Olympus Fluoview 500 confocal microscope using ImagePro version 5 to acquire and process images (Melville, NY, USA). Auto-fluorescence was observed over the entire visible spectrum and into the infrared. Images were acquired simultaneously in three separate channels using filter sets designed for Cy3, Cy5 and fluorescein (excitation 488, 543 and 633 nm, emission filters 505–525, 560–600, and >600 nm).

Amino acid analysis

Glass beads (50–100 mg) containing cement disks were hydrolyzed in 100 µl 6 mol l⁻¹ HCl and 10% phenol *in vacuo* at 110°C. The acid hydrolysates were flash evaporated to dryness with three changes of de-ionized water and two with 100% methanol using a Buchler vacuum evaporation unit set at 60°C. Amino acid analysis following acid hydrolysis was performed as described previously (Waite, 1991). Briefly, a Beckman System 6300 Autoanalyzer equipped with a Na HPLC ion exchange column (Beckman Coulter #338076; Fullerton, CA, USA) was used. The elution program was 85 min long to allow full separation of DOPA from Leu. Amino acid detection based on derivatization by ninhydrin was monitored at 570 and 440 nm, and absorbances were processed by HP ChemStation for LC [Hewlett-Packard Rev A.06.03(509); Wilmington, DE, USA] in external standard mode using a standard amino acid mixture (Sigma # A9 531; St Louis, MO, USA).

Results

When individual *P. californica* in a short segment of their tube were laid on a bed of glass beads (0.5 mm diameter) they worked compulsively to rebuild their tube by cementing the available glass beads to the tube's anterior end (Jensen and Morse, 1988). In a 24 h period, a typical adult animal extended its tube by up to 1 cm with glass beads. The cement of freshly deposited glass beads was creamy white. Over a period of about 6 h the cement spots became distinctly reddish brown in color. Old cement spots (>48 h) were dark reddish brown. SEM examination of cemented glass beads less than 24 h old revealed cement disks with uniform diameters (about 200 µm) that were positioned at uniform distances from one another on the beads (Fig. 1A). When tubular sections of cemented glass beads were broken into individual beads in the hydrated state, adhesive failure at the interface with the glass surface consistently left the cement disk intact on one of the glass beads (Fig. 1A). Conversely, when intact sections of cemented glass beads were frozen and lyophilized before being separated, cohesive failure frequently occurred within the glue disks. The interior of the cement disks had the appearance of a closed-cell solid foam enclosed by a porous skin (Fig. 1B,C). The diameter of the cells ranged from about 0.25 to 4 µm. More than a dozen fractured specimens examined by SEM all had similar cellular structures with similar ranges and distributions of cell diameters.

To verify that the foam-like structure was not an artifact of the SEM processing and fracturing procedure, we took advantage of the observation that the cement disks auto-fluoresced across the visible spectrum, strongest in the red, to examine hydrated and unprocessed cement disks by laser scanning confocal microscopy. Optical sections through intact cement disks (Fig. 2) revealed a cellular structure with closed

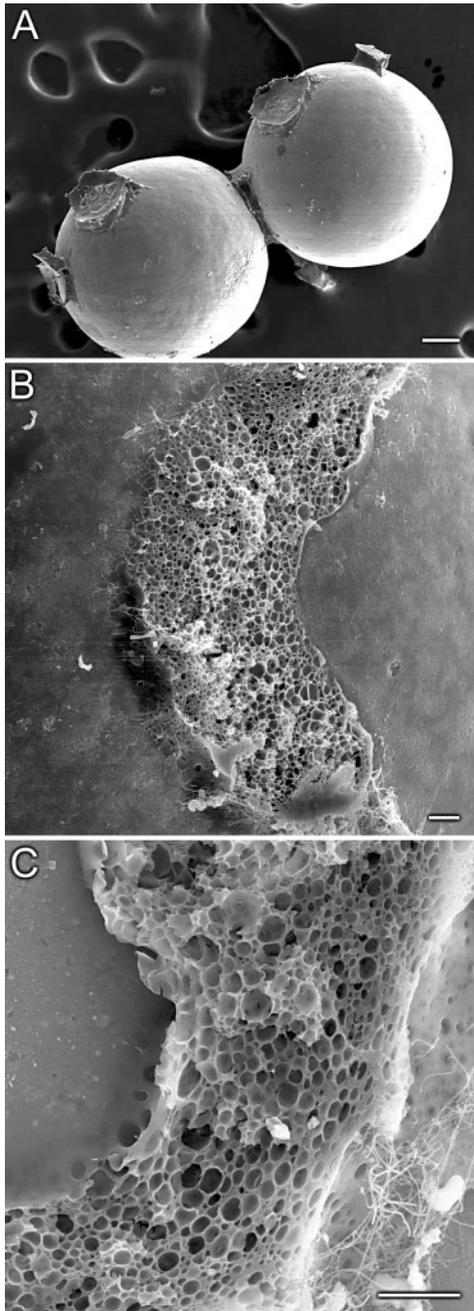


Fig. 1. Representative SEM micrographs of cement disks on 0.5 mm glass beads removed from a reconstructed *P. californica* tube. (A) Intact cement disks on beads separated from a section of reconstructed tube in a hydrated state. Scale bar, 100 μm . (B,C) Fractured cement disks on beads separated after lyophilization. Scale bars, 10 μm .

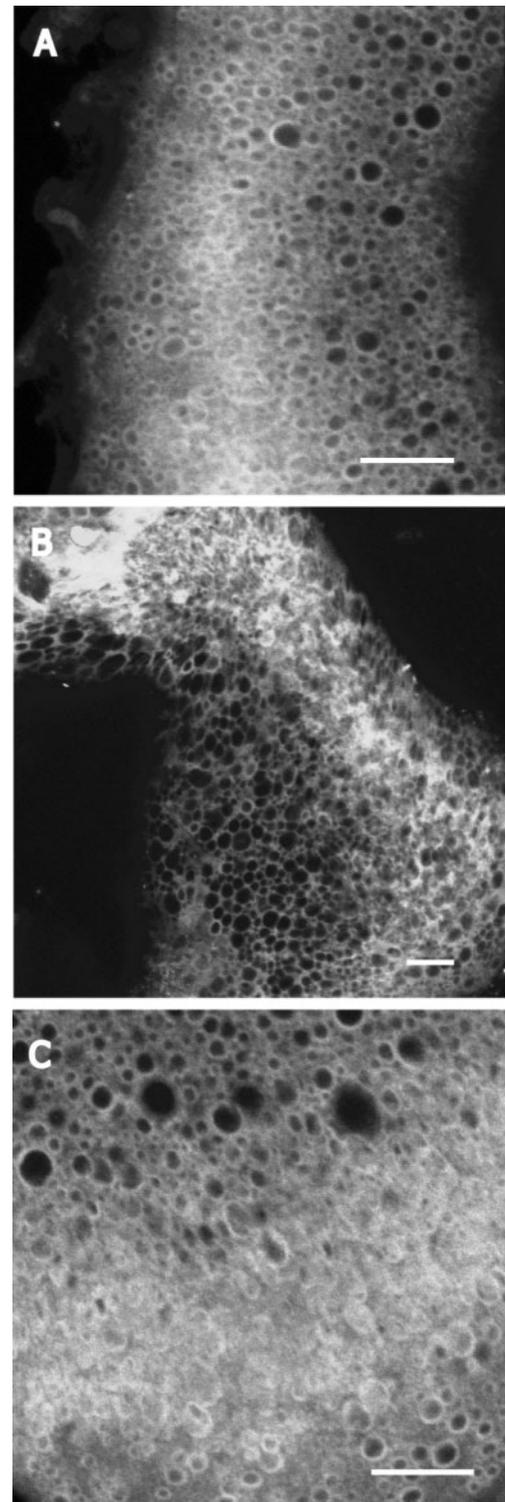


Fig. 2. Representative optical cross-sections through three separate intact cement disks in seawater by laser scanning confocal microscopy. The cement was visualized by auto-fluorescence. (A) Overlay of images acquired with FITC and Cy5 filter sets; (B,C) overlay of images acquired with FITC, Cy3 and Cy5 filter sets; excitation 488, 543 and 633 nm, emission filters 505–525, 560–600, and >600 nm. Scale bars, 10 μm .

cells and a cell-size distribution similar to the cellular structure observed by SEM. The continuous phase was auto-fluorescent and the discontinuous phase was non-fluorescent. In total, four separate cement disks were optically sectioned and found to have similar cellular structures.

The elemental composition of the cement was examined by energy dispersive spectrometry (EDS). Cemented beads were washed in excess de-ionized water or 0.5 mol l^{-1} NaEDTA (pH 8.0). In Fig. 3A, an EDS spectrum from a region of a cement disk washed in water was overlaid onto an EDS spectrum acquired from an adjacent bare region of the same glass bead that had approximately the same orientation to the EDS detector. The major elements consistently observed in relatively greater proportion in the cement than in the glass, in the order of energy, were C, N (not labeled), O, Mg, P and Ca. Convincing peaks above background for other elements, including Fe and Al, were not consistently observed. Eight cement disks had qualitatively similar elemental compositions relative to an adjacent bare region of the silica bead. Overlaid EDS spectra from cement disks and beads washed with 0.5 mol l^{-1} NaEDTA demonstrated that the relative amount of

phosphorus was undiminished by washing with EDTA (Fig. 3B), suggesting that the phosphorus was part of the covalent structure of the cement rather than in a mineral form that would have been dissolved by EDTA. Carbon, N and O were also undiminished by washing with EDTA, as expected for covalent elements of the protein cement. Magnesium and Ca, conversely, were almost entirely extracted and exchanged with Na. Similar spectra were observed with five separate cement disks washed with EDTA. This suggests that the interior of the cement disk is accessible to the external solution. No other effects of EDTA on the cement disks were observed by light microscopy.

Spatial mapping of the EDS results show that P, Mg and Ca are indeed localized within the cement disks (Fig. 4). After washing with EDTA, P remains in the cement disk while Mg and Ca are exchanged for Na (Fig. 5). The Mg and Ca are most likely complexed to the cement, as opposed to being trapped in the cells of the cement, because they were not removed by thorough washing with de-ionized water.

During amino acid analysis of cement that was acid hydrolyzed off the glass beads, an acidic amino acid eluted at 3.2 min, about 7 min before the first standard amino acid (4-hydroxyproline). Two lines of evidence suggested that the early non-standard amino acid was O-phosphoserine (pSer).

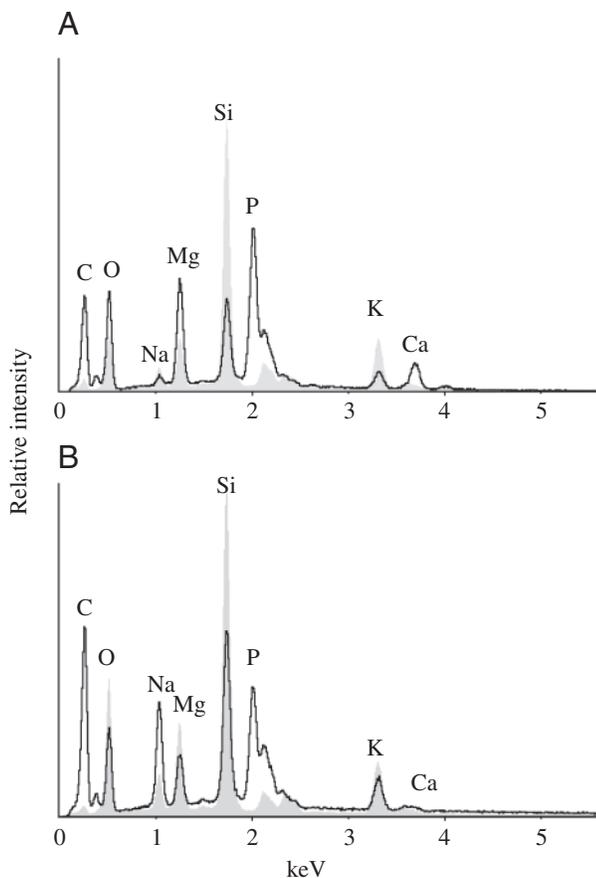


Fig. 3. Representative EDS spectra. (A) From a glue disk washed in de-ionized water (open curve). From a bare region of the same bead adjacent to the glue disk (grey-filled peaks). (B) From a glue disk washed with 0.5 mol l^{-1} NaEDTA, pH 8.0 (open peaks). Bare surface of silica bead (grey-filled peaks). keV, kiloelectron volts.

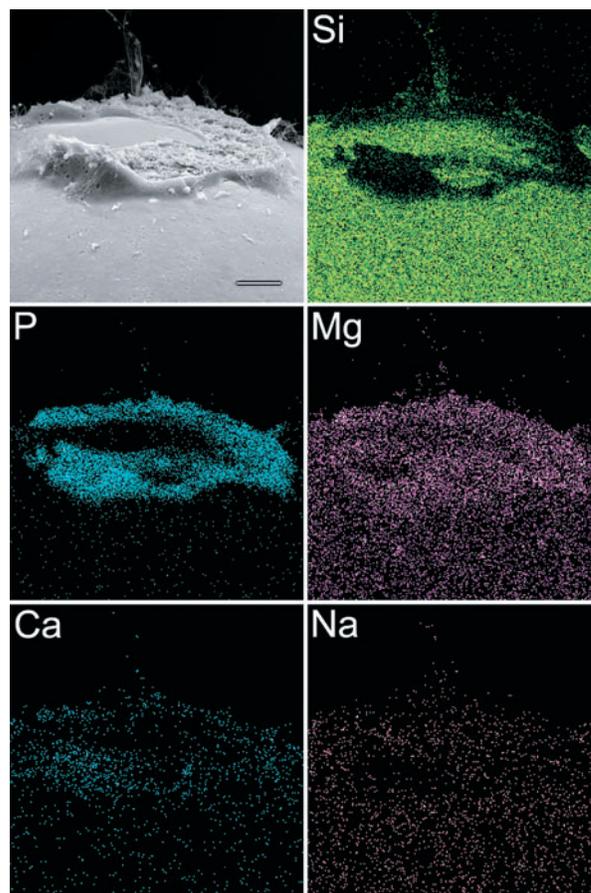


Fig. 4. SEM and EDS spatial maps of a glue disk washed with de-ionized water. Scale bar, $25 \mu\text{m}$.

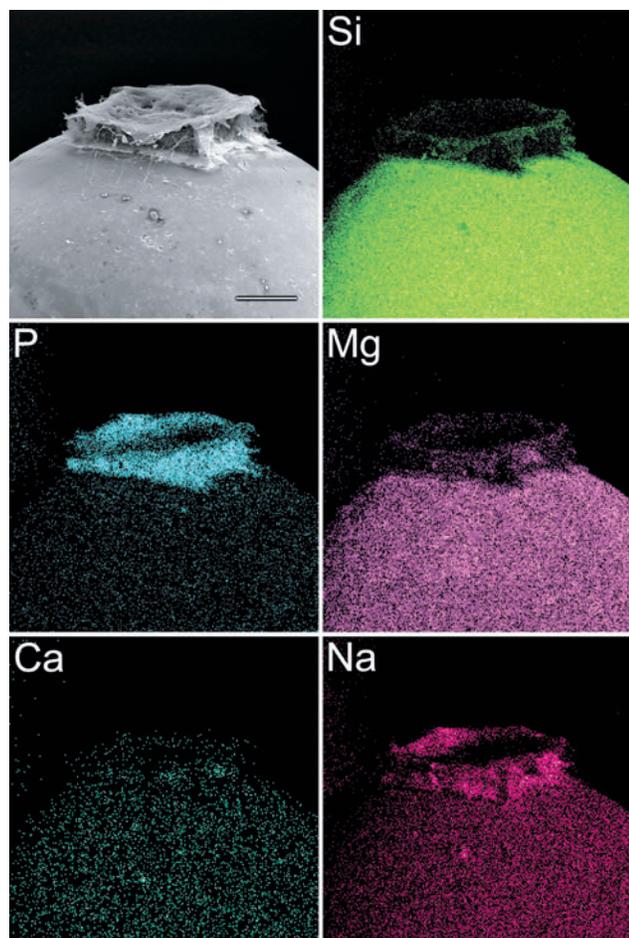


Fig. 5. SEM and EDS spatial maps of a glue disk washed with 0.5 mol l^{-1} NaEDTA. Scale bar, $50 \mu\text{m}$.

First, an O-phosphoserine standard eluted at the same time (3.2 min). Second, timed hydrolyses of the cement revealed a decrease in the 3.2 min peak and a proportionate rise in the Ser peak over time, reflecting the hydrolytic conversion of pSer to Ser (Fig. 6). Together, pSer and Ser accounted for close to 30 mol% of the cement amino acid residues. After a 1 h hydrolysis, 85% of the Ser was in the form of pSer (Fig. 7). Extrapolating to zero hydrolysis time, we estimate that 95% or more of the Ser is phosphorylated in the cement. Therefore, the bulk of the P observed in the cement by EDS was in the form of O-phosphoserine.

Discussion

Phragmatopoma californica tube cement has an internal structure reminiscent of a solid foam with closed cells that is covered by a less porous skin (Fig. 1). In this respect, it closely resembles the foam-like structure of the byssal adhesive plaques of mussels (Benedict and Waite, 1986; Tamarin et al., 1976). The foam-like cement of a tubicolous polychaete was noted as early as 1903 (Fauvel, 1903), who described the ‘cement bulbous’, or ‘bubbly cement’ of a pectinariid

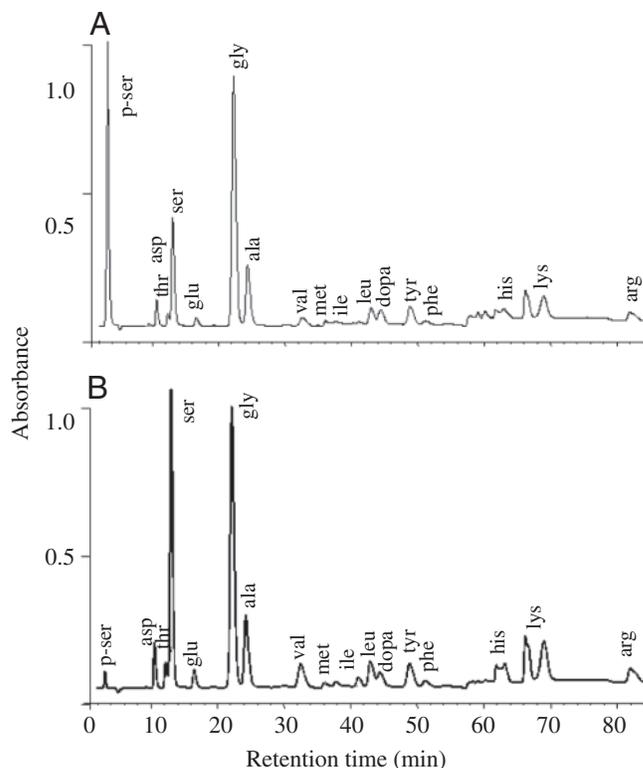


Fig. 6. Amino acid chromatograms from acid hydrolysis time course. (A) 3 h, (B) 28 h. Loss of pSer (3.2 min) is accompanied by gain of Ser (13.2 min).

polychaete. Like the sabellariidae, the pectinariidae cement adventitiously gathered particles into a solitary tube, but do not form colonies.

Based on sound engineering principles, foamed adhesives offer several benefits for tubeworms. First, the foam-like structure would increase the cement’s elasticity and toughness – the amount of energy a material can absorb before failing (Gibson and Ashby, 1997). The more flexible cement junctions would absorb and dissipate the energy of the impinging surf in the inter-tidal environment to minimize damage to the tube, like foam packing material. A related benefit is the demonstrated crack-stopping behavior of foams. Second, the cellular structure of the cement would save material and metabolic energy. If the cohesive strength of the solid cement were much greater than the adhesive strength of the cement to the substrate, then the extra material in a solid cement would be wasted. Matching the adhesive and cohesive strengths of the cement is more economical. Third, a foam structure could minimize the abruptness of the elastic modulus mismatch between the rigid particles and the flexible cement. A gradient in the cell size from the interface with the particle to the center of the cement, with the smallest cells being close to the interface, would create a gradient in the elastic modulus with the highest cement modulus being at the interface.

Regarding composition, the cement appears to consist of a set of polyelectrolytes with opposite net charges at physiological pH. The previously identified precursor proteins

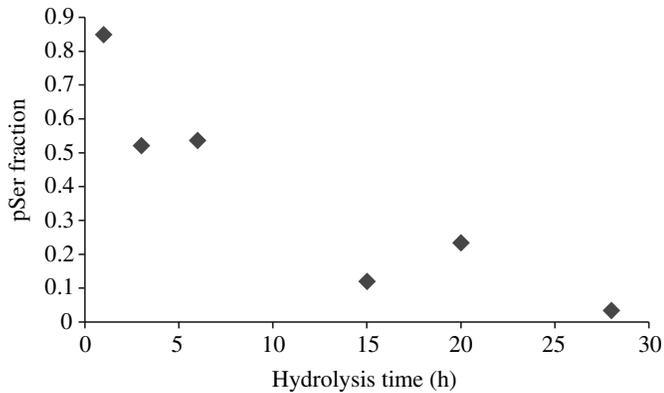


Fig. 7. pSer fraction (pSer/Ser+pSer) during cement acid hydrolysis time course.

Pc1 and Pc2 are positively charged. They contain relatively high percentages of basic residues (approximately 15 mol% each), few acidic residues and, accordingly, their isoelectric points are predicted to be 9.75 and 9.95, respectively (Waite et al., 1992). They also contain almost 10 mol% DOPA residues each. The other major cement protein (or proteins) whose soluble precursor has yet to be isolated is negatively charged. It contains a high proportion of acidic pSer. All in all, the cement contains about 19% basic residues and about 30% acidic residues, predominantly pSer. It also contains significant quantities of complexed divalent cations. The Ca^{2+} and Mg^{2+} of *P. californica* cement are not likely to be in a phosphate mineral form, as has been suggested of *P. koreni* cement (Truchet and Vovelle, 1977a), because EDTA washed away the vast majority of Ca^{2+} and Mg^{2+} while not significantly diminishing the amount of phosphorus. If the phosphorus occurred in mineral form, it too would have been dissolved when Ca^{2+} and Mg^{2+} were sequestered by EDTA. In *Sabellaria alveolata* (Gruet et al., 1987) and in *Pectinaria koreni* (Truchet and Vovelle, 1977a), and therefore almost certainly in *P. californica*, high concentrations of Ca^{2+} and Mg^{2+} are present within secretory granules of the cement glands.

The mechanisms by which polychaetes produce the solid cellular foam structure in their cement is of considerable biological and technological interest. It is not likely to involve the blowing of a molten polymer as in the manufacture of Styrofoam™ and polyurethane foams (Shutov, 1986). Such processes require a gaseous blowing agent and, practiced underwater, would produce a material with undesirable buoyancy. Rather, the foam-like structure is probably formed from two interspersed liquid phases. A possible mechanism that would account for phase separation during cement formation is suggested by earlier cytological observations, by the composition of the tube cement, and by classic studies of protein colloid chemistry. We consider each of these in turn. Electron micrographs of thin sectioned cement glands of *P. koreni* (Truchet and Vovelle, 1977a) and *S. alveolata* (Vovelle, 1965) revealed polymorphic secretory granules originating in distinct cell types. Some of the secretory granules, still deep within the cement glands, clearly had the

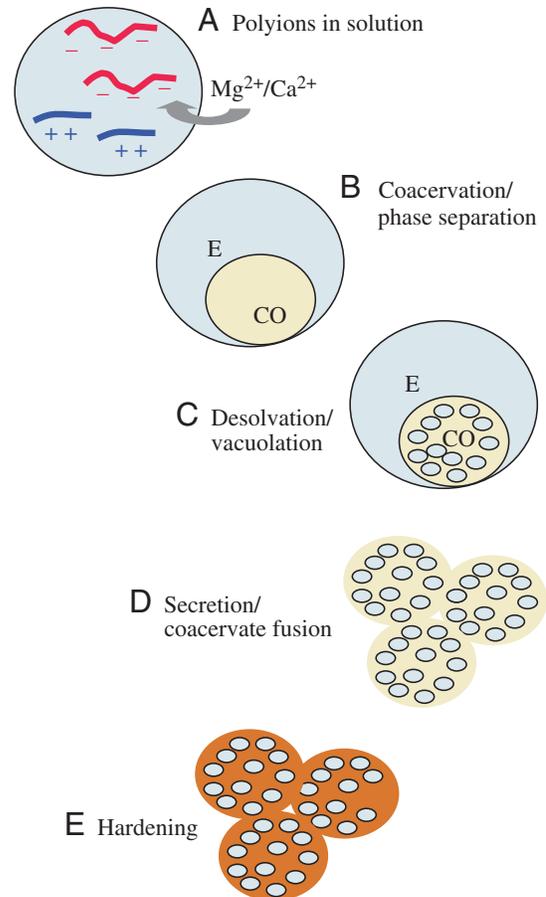


Fig. 8. Complex coacervation model of foam cement formation. (A) Macroanion (red) and macrocation (blue), pH 5. Mg^{2+} and Ca^{2+} are pumped in to neutralize excess polyanion charges. (B) Neutralization leads to phase separation of the coacervate (CO) from the equilibrium solution (E). CO is an enriched blend of anion, cation and Mg^{2+} . Both phases are fluid although CO is viscous. (C) As volume of CO phase increases and CO desolvation proceeds, more vacuoles of E form within CO instead of diffusing to E phase. (D) Upon secretion into seawater at pH 8, electrostatic interaction of Mg^{2+} and Ca^{2+} with phosphate groups becomes ionic due to low solubility, increasing the viscosity of CO. (E) Oxidation of Dopa to quinones leads to cement solidification by cross-linking.

'bubbly' appearance of the deposited cement. This suggests that the process that leads to phase separation in the cement may begin during the maturation of the secretory granules. A common feature of secretory granule formation is the condensation of a polyanionic matrix through crosslinking by multivalent cations. Two well-studied examples are the condensation in mast cells of negatively charged heparin glycosaminoglycan by histamine, a divalent cation (Nanavati and Fernandez, 1993; Verdugo et al., 1987); and the condensation in goblet cells of the negatively charged mucin glycoprotein by Ca^{2+} (Verdugo, 1990). It follows that condensation of the cement into dense secretory granules may occur by interaction of the anionic polyphosphoserine protein with polycationic Pc1, Pc2, Ca^{2+} and Mg^{2+} . Bungenberg de

Jong (Bungenberg de Jong, 1949a,b) described numerous examples of the spontaneous separation of an aqueous solution of two more oppositely charged polyelectrolytes into two immiscible aqueous phases – a dilute equilibrium phase and a denser solute-rich phase – in a process referred to as complex coacervation. Complex coacervate systems have the following characteristics. (1) Both phases are predominantly water. The coacervate phase is an isotropic liquid containing amorphous associative particles that move freely relative to one another. (2) Coacervation occurs when the charges of the polyelectrolytes are balanced. Coacervation is therefore pH dependent, occurring to the maximum extent at the pH where the solution is electrically neutral; ionic strength dependent, since shielding of charges can change the charge balance of the system; and dependent on the ratio of polyelectrolyte concentrations. (3) Complex coacervation usually involves at least one flexible random coil polymer. The flexibility may mediate associative interactions between the colloidal aggregates of the liquid coacervate phase. (4) Coacervates occur in diverse morphologies, including, under certain conditions, foam structures with transient water-filled vacuoles (Bungenberg de Jong, 1949b).

Based on the above considerations, we propose that phase separation of the sabellariid cement may occur as a result of a complex coacervation process during secretory granule maturation (Fig. 8). The complex coacervation occurs between Pc1 and 2 (polycations), the pSer-rich protein (polyanion) and $\text{Ca}^{2+}/\text{Mg}^{2+}$. These components are mixed in the cement glands in a ratio that is electrically neutral and thus phase separate (Fig. 8A,B). Water (E) is expelled from the coacervating ions as they condense and desolvate. Initially, when the coacervate droplets are small, the excluded water will diffuse directly into the E phase (Fig. 8B). As the size and viscosity of the coacervate droplets increases, the excluded water will become increasingly entrapped in the coacervate phase as discontinuous vacuoles (Fig. 8C).

Bungenberg de Jong (1949a) referred to the process as ‘vacuolation’, and this could well give rise to the cellular foam structure of the cement. Our model suggests, as a parsimonious explanation for the origin of the sabellariid cement, that the ordinary secretory process may have been adapted to the production of a foam cement. This adaptation may have occurred through natural selection of the type of polyanionic secretory matrix in the cement gland. For example, a polyphosphate matrix may coacervate more readily than the more common polycarboxylate matrices because at the pH of the secretory vesicle (pH 5) the polyphosphate (pK_{a1} 2) would be more charged than the polycarboxylate (pK_a 4). Likewise, a polysulfate matrix such as the heparin glycosaminoglycan matrix of mast cells would likely be too bulky and stiff to efficiently form coacervates. Additional parameters that may have been adjusted by natural selection to maximize coacervation and optimize cell dimensions and size distributions include the charge density of the polyions, the flexibility of the polyions, and of the ratio of polyions to divalent cations.

During secretion, the vacuolated cement droplets apparently

coalesce (Fig. 8D) into a single cohesive cement disk with a diameter of about 200 μm . Secretion is accompanied by a jump in pH from ~ 5 in the secretory granule to 8.2 in seawater that could trigger two events in the coacervate. First, deprotonation of histidine residues ($\text{pK}_a \sim 6.5$) and the consequent loss of the positive charge would free up their phosphate counterions to interact with $\text{Ca}^{2+}/\text{Mg}^{2+}$ cations. Second, the nature of the interactions between $\text{Ca}^{2+}/\text{Mg}^{2+}$ and phosphate groups would change from coulombic interactions between solvated ions to bonds more akin to ionic bonds in an insoluble salt, the effect of which would be to harden spontaneously and solidify the cement (Fig. 8D).

Another environmental change accompanying cement secretion is exposure to a high concentration of monovalent Na^+ cations ($>0.5 \text{ mol l}^{-1}$). The large excess of monovalent Na^+ could displace some of the multivalent cations crosslinking the polyphosphoserine matrix, leading to the rapid absorption of water (Verdugo et al., 1987). This sudden thirst may contribute to removing water at the interface with the substrate to facilitate the underwater adhesion of the cement proteins to the substrate, particularly the DOPA residues of Pc1 and 2 (Waite, 2002). The swelling due to the absorption of water would also increase the volume and more importantly the adhesive area of the cement disk. With regard to adhesion, the polyphosphoserine protein may also play a direct role in adhesion of the cement to calcareous substrates. Several phosphoproteins have been shown, or suggested, to bind strongly to calcareous minerals. These include the saliva protein statherin, which binds to hydroxyapatite (Long et al., 2001); osteopontin, a matrix protein of bones (Boskey et al., 1993) and calcareous kidney stones (Kohri et al., 1992); phosphoryn, the extremely acidic phosphoprotein of dentin (Ritchie and Wang, 1996), and mefp-5 from the adhesive pad of mussel byssal threads (Waite and Qin, 2001).

The presence of DOPA residues in Pc1 and Pc2 suggest that the cement is, at least in part, hardened by diDOPA crosslinks. Quinone-tanning occurs in a wide range of DOPA-containing structural proteins (Waite, 1995). The mechanism of oxidative crosslinking between DOPA residues has been most extensively studied in the DOPA-containing proteins of the mussel byssal thread (Burzio and Waite, 2000; McDowell et al., 1999). Since the phenolate form of DOPA is more redox active than the protonated form, DOPA crosslinking would be accelerated by the shift to the elevated pH of seawater. That the bulk of the quinone tanning probably occurs after deposition of the cement onto the particle is suggested by the observation that freshly deposited cement is white and creamy but turns brown within a few hours. Solidification through covalent cross-linking after secretion of the complex coacervate would prevent separation of the two dispersed aqueous phases, locking in the final solid foam structure of the cement, while providing its ultimate cohesive strength.

In conclusion, the robust underwater adhesion to diverse substrates, the composition, and the foam-like structure of its cement, hint at a sophisticated materials engineering practice of *P. californica*. There remain many questions, particularly

regarding the details of the molecular structure of the cement, the cytological location of individual cement proteins, and changes in the structure during and after the secretion process. In pursuing these questions, it is likely that there is much that *Phragmatopoma californica* will teach us about designing adhesives.

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