J|A|C|S COMMUNICATIONS

Published on Web 08/19/2010

Oysters Produce an Organic−Inorganic Adhesive for Intertidal Reef Construction

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Abstract: Coastal ecosystems rely upon oyster reefs to filter water, provide protection from storms, and build habitat for other species. From a chemistry perspective, few details are available to illustrate how these shellfish construct such extensive reef systems. Experiments presented here show that oysters generate a biomineralized adhesive material for aggregating into large communities. This cement is an organic−inorganic hybrid and differs from the surrounding shells by displaying an alternate $CaCO_3$ crystal form, a cross-linked organic matrix, and an elevated protein content. Emerging themes and unique aspects are both revealed when comparing oyster cement to the adhesives of other marine organisms. The presence of cross-linked proteins provides an analogy to mussel and barnacle adhesives whereas the high inorganic content is exclusive to oysters. With a description of oyster cement in hand we gain strategies for developing synthetic composite materials as well as a better understanding of the components needed for healthy coastal environments.

Up through the 18th century intertidal oyster reefs provided a major determinant of sea life along the Eastern Seaboard of the United States. Billions of shellfish aggregated into reef structures tens of meters deep and several square kilometers in area.[1] In doing so, oysters created habitat for other species, filtered large volumes of water, and protected the coast from storms.[2] Since the late 1800s overfishing, pollution, and disease have reduced stocks substantially.[2] During this time oyster harvests from once-bountiful locations such as the Chesapeake Bay have declined by 98% or more.[1] A great deal of effort is currently being invested to reintroduce oysters to their earlier habitats.[3]

Despite the vital role played by oysters in maintaining robust coastal ecosystems, we know few details about the chemistry of how these shellfish build reefs. The ability of select, mature oysters to produce cement has been examined at a microstructural level.[4] Little data are available to describe reef assembly by the common Eastern oyster, *Crassostrea virginica*, a shellfish of great environmental, economic, and culinary impact. Experiments presented below show that oyster reef construction is based upon the animals generating an organic−inorganic hybrid material. This adhesive differs significantly from the shells on either side.

Clusters of Eastern oysters were collected at the Baruch Marine Field Laboratory, South Carolina, USA, where complete intertidal reef structures can still be found (Figure 1A). Within aggregated clusters we focused on attached animal pairs (Figure 1B). Cross

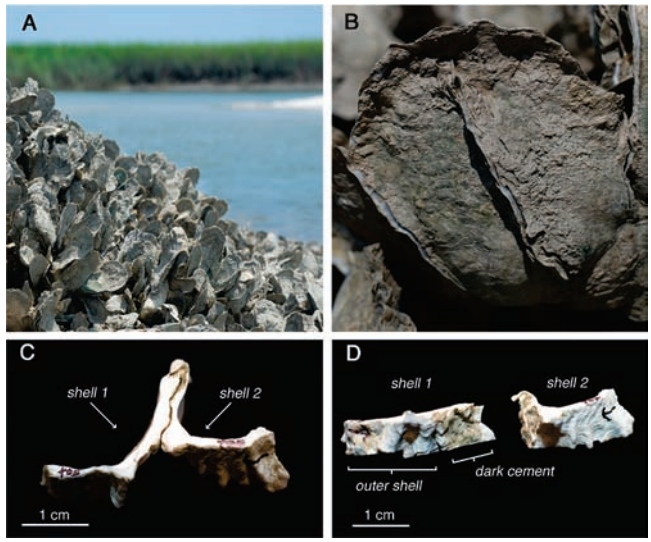

Figure 1. (A) Oysters forming an intertidal reef. (B) Two oysters cemented together. (C) An interface between two attached oysters, exposed after cutting to reveal a cross section. (D) Same sample as in (C) after separation of the two shells. Note the dark cement.

sections were cut, thereby exposing the interface between animals. Figure 1C shows such a sample in which the shell of one animal can be seen affixed to that of another. A band of gray material is visible in the center, darker than the bulk and outer shells. Attached shells were separated with a metal punch run into the cement region of cross sections. Cement could then be scraped off the shell surfaces. For comparison, samples of outer shell (without cement) were also scraped and collected to yield powders. Likewise, pseudonacre from the shell interior was isolated (Figure S1).

Separation permitted examining the composition of oyster cement relative to the outer shell and inner pseudonacre. Predrying (80 °C, vacuum, 2 days) of each sample showed <2% mass loss, indicating low water contents. Neither extensive treatment with acid nor a strong chelator could render the cement fully soluble, thereby indicating the presence of cross-linked material. However thermogravimetric analysis (TGA) could be used to ascertain the relative mass loss from water, organics, and inorganics upon heating in each predried sample, along with a $CaCO_3$ control. The $CaCO_3$ forms nonvolatile CaO (56%) at high temperatures and provides a limit for mass loss in calcareous samples. Results from TGA mass loss, summarized in Table S1, show that the adhesive (3.1%), outer shell (0.5%), and pseudonacre (0.5%) are all low in water.

The inorganic content of the adhesive material (20.0%) was less than that of the shell (30.7%) and pseudonacre (32.0%). The adhesive also exhibited notably more organics (11.2%) than either the outer shell (2.0%) or pseudonacre (1.3%). These findings of

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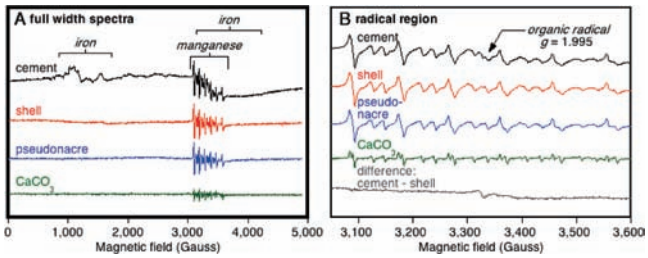

Figure 2. (A) EPR spectra of oyster cement, outer shell, and pseudonacre along with a $CaCO_3$ control. (B) Close-ups of the region containing the 16 line Mn^{2+} signals and organic radicals.

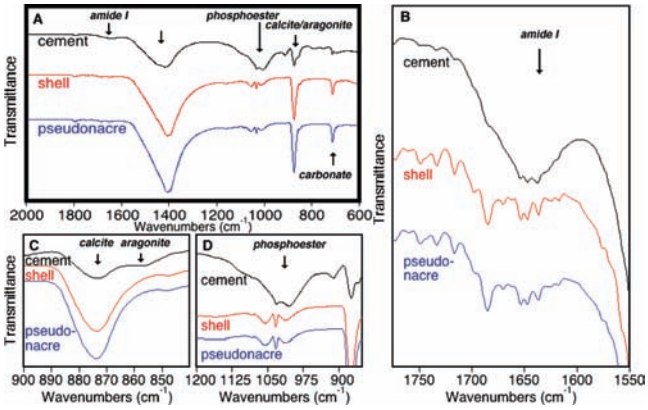

Figure 3. (A) IR spectra of oyster cement, shell, and pseudonacre. Expansions are shown for regions of (B) protein amide I, (C) calcite/aragonite, and (D) phosphate esters.

~1−2% organics in shell and pseudonacre fit well within the limits of reported values at 0.3−3%.[5] High organic levels in oyster adhesive do not appear to result from macromolecular or cellular fouling given that similarly elevated organics were not observed on outer shells exposed to salt water. Overall, shell and pseudonacre are similar in composition whereas the adhesive differs.

Separated adhesive, outer shell, and pseudonacre were examined by electron paramagnetic resonance (EPR) spectroscopy (Figure 2). The conspicuous 16 line signals arise from trace Mn^{2+} typical of all calcium rich samples,[6] including a $CaCO_3$ control (Figure 2). For the cement, a 17th resonance is visible ($g = 1.995$, 3333 G) where organic radicals are found (Figure 2B).[7] This potential radical signal is more apparent in a difference plot of the shell spectrum subtracted from that of the adhesive (Figure 2B). Most evident in the cement full EPR spectrum (Figure 2A) are resonances in the ~800−1700 G ($g \approx 8.3-3.9$) region and a broad signal centered around 3800 G ($g \approx 1.8$), manifested as a baseline drop, both of which are characteristic for iron (high-spin Fe^{3+}, low-spin Fe^{3+}, or Fe−Fe coupling).[8] In the case of mussel adhesive, iron has been shown to induce oxidation of proteins to yield radical species and subsequent curing of the material.[9] Cross-linked proteins also play a central role in formation of barnacle cement.[10]

Infrared (IR) spectroscopy further emphasized composition differences between the adhesive material versus the outer shell and pseudonacre (Figures 3 and S2). Strong absorbance at 1390, 872, and 711 cm^{-1} along with comparison to a $CaCO_3$ control (Figures 3A and S2) shows that the adhesive, shell, and pseudonacre are all high in carbonate, correlating well with findings of high inorganics from the TGA data (Table S1). The shell[11,12] and pseudonacre[12,13] of *C. virginica* are known to be predominantly calcitic $CaCO_3$ with a minor fraction of aragonite. Consistent with these reports, the IR spectra of shell and pseudonacre show absorbance predominantly at 876 cm^{-1}, specific for calcite, and little for aragonite at 858 cm^{-1} (Figure 3C).[14] Here, too, the adhesive differs appreciably from the shell on either side with a prominent aragonite component visible. From ratios of peak intensities, the $CaCO_3$ of oyster cement appears to be ~1/3 aragonite and ~2/3 calcite.

Further differences contrasting oyster adhesive with shell and pseudonacre were found in the ~1640 cm^{-1} protein amide I band. The adhesive displays more intense protein absorption (Figure 3B) as well as weaker carbonate absorption (Figure 3A). Consequently the protein/carbonate ratio is significantly higher for the adhesive than that found in the shell and pseudonacre. Absorbance in the 1150−950 cm^{-1} range is strongest for the adhesive (Figure 3D) and can be assigned to phosphoesters.[15] Evidence for phosphoesters may indicate that the adhesive proteins are phosphorylated and providing a matrix for the $CaCO_3$.[16] The main organic component of the *C. virginica* shell matrix is known to be acidic phosphoproteins.[17] Phosphoserine-containing proteins are becoming a biomaterials motif, with examples found in the adhesives of mussels, tube worms, and sea cucumbers.[18]

Taken together, data presented here show that oysters produce an organic−inorganic hybrid cement for aggregating into reef communities. This adhesive differs from the shells to which it is attached. The organic component of oyster cement is elevated ~5× relative to shell, although the inorganic fraction predominates. More specifically, spectroscopic results indicate that oysters generate an organic matrix of cross-linked, phosphorylated protein to harbor the inorganic component of their cement. Themes in marine biological materials may be emerging, with proteins known to be central to the adhesives of mussels,[19] barnacles,[10] and, now, oysters. Each system, however, has unique aspects. Mussel adhesive[19] as well as the mature, primary and fresh, secondary cements of barnacles[10] are all largely protein. The water content of primary barnacle cement, at ~20−50%, is ~10× that found here for oysters.[20] This mature adhesive from oysters appears to be more of an inorganic cement than the primarily organic, hydrated glues of mussels and barnacles. By revealing the nature of this bioadhesive we hope to provide blueprints for the design of biomimetic materials, aid development of adhesion-inhibiting antifouling surfaces, and illustrate the workings of healthy coastal ecosystems.

Acknowledgment. Neeraj Gohad and Andrew Mount imparted insights through many helpful discussions. Experimental assistance was provided by Jessica Burkett, Steven Cummings, Michael Everly, Tong Ren, and Alex Ribbe. We appreciate financial support from the Office of Naval Research and the National Science Foundation.

Supporting Information Available: Experimental methods, IR spectra, and TGA data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA104996Y