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Cement Precursor Proteins of the Reef-Building Polychaete *Phragmatopoma californica* (Fewkes)

J. Herbert Waite,*,‡ Rebecca A. Jensen,§ and Daniel E. Morse*,§

College of Marine Studies, University of Delaware, Lewes, Delaware 19958, and Marine Biotechnology Center and Department of Biological Sciences, University of California, Santa Barbara, California 93106

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ABSTRACT: Two distinctive 3,4-dihydroxyphenyl-L-alanine- (DOPA-) containing proteins (Pc-1 and Pc-2) have been isolated and partially characterized from the thorax of the reef-building sabellariid *Phragmatopoma californica*. They are the first such reported from the phylum Annelida. The proteins are presumed to be soluble precursors of the quinone-tanned cement used to bind particulate materials in the construction of the tubes that serve as habitats for the worms. The proteins have apparent molecular weights ranging from 18 000 to 20 000 and isoelectric point ≥ 8.0. Both proteins consist of repeated sequence motifs in their primary structure. Pc-1 has repeats of {XGGY*GY*GAK} where X = V, L, I, AA, or KV, and Y* is DOPA or tyrosine. Pc-2, in contrast, appears to have repeats of $\{X_1\text{-}[GGY^*]_n\text{-}[GA]_m\text{-}X_2\text{-}[HP(A)V]_p\text{-}HK\}$ where X_1 can be AL, A, or F; X_2 can be WG or absent; n and m can be 1 or 2, and $p = 0$–2. Both protein families appear to share the same C-terminal sequence ALGGY*GAGA. Of the DOPA-containing proteins characterized from other phyla, *Phragmatopoma* cement precursors most resemble those from the liver fluke *Fasciola hepatica* and the mussel *Trichomya hirsuta*.

The gregarious marine sabellariid polychaetes build massive reef-like mounds which consist of a honeycomb of contiguous tubes each with a permanently resident polychaete (Hartman, 1944; Sisson, 1986). The tube walls are constructed in a manner that closely resembles stone masonry. The polychaete collects passing particulate material and debris (average diameter 500 μm in *Sabellaria alveolata*) from the water column

‡ University of Delaware.
§ University of California.

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and, after tactile examination, cements it onto the growing edge of the burrow wall using an organic mortar derived from cement glands in the thorax (Vovelle, 1965; Gruet et al., 1987). While the strength of these tubes has yet to be measured, their durability and location in the turbulent intertidal zone suggest a robust construction.

In materials science, a composite material is defined as consisting of two or more physically distinct and mechanically separable materials and having properties that are superior and possibly unique in some respects to the properties of the individual components (Hull, 1981). The two distinct materials in the tubes (organic mortar and solid filler consisting of sand, bits of shell, etc.) reflect just one example of nature's innumerable composite materials. Since the design of durable man-made composites is plagued by the presence of moisture at interfaces between the different materials in a composite (Bowditch & Stannard, 1984; Hull, 1981), the underwater fabrication of composite tubes by sabellariids seems an ideal model of how nature circumvents the subversive interfacial effects of water. The organic mortar used by the West Coast sabellariid *Phragmatopoma californica* is of particular interest because of its (*1*) ability to bond to wet surfaces, (*2*) versatility in bonding to a variety of particulate substrata, and (*3*) effectiveness at low mortar-to-filler weight ratios (Jensen & Morse, 1984; Gruet et al., 1987). Recently, the cement was identified as proteinaceous and particularly enriched in the amino acids serine, glycine, and lysine (Jensen & Morse, 1988). In addition, significant 3,4-dihydroxyphenyl-L-alanine (DOPA) was detected in the cement (Jensen & Morse, 1988), suggesting a resemblance with the byssal adhesives of marine mussels (Waite, 1987). In the present study, two cement gland proteins have been isolated from *P. californica* thoraces and characterized.

Experimental Procedures

Extraction of Cement Proteins. The cement glands of *P. californica* are prominently located in the thorax of the adult worm. Worms were carefully removed from tubes fragmented from reef material obtained from collection sites near Santa Barbara, CA. Thoraces were dissected from 100–200 worms and frozen at −80 °C until further use. Cement proteins were directly extracted from thoraces (wet weight) by homogenization (Dounce ground-glass tissue grinder) at 4–7 °C in 5–10 volumes of 4 M urea with 5% acetic acid and 10 μM leupeptin. The extracts were centrifuged at 35000*g* for 30 min at 5 °C (±2 °C). The supernatant was carefully recovered and adjusted first to 10% (w/v) and then 20% ammonium sulfate. After each addition of ammonium sulfate, the supernatant was stirred for 30 min at 6 °C and then centrifuged at 20000*g* for 30 min at 5 °C. The precipitate from 20% ammonium sulfate was redissolved in 2 mL of 5% acetic acid with 4 M urea and dialyzed against 5% acetic acid. The volume was reduced to about 3 mL by ultrafiltration under 4 kg cm^{-2} of nitrogen using an Amicon PM-10 filter, and the concentrate was applied to a column (2 cm × 75 cm) of Sephadex G-75 eluted with 5% acetic acid containing 4 M urea. Fractions were directly monitored at 280 nm and assayed colorimetrically at 500 nm for DOPA (Waite, 1983). DOPA-rich fractions were pooled and concentrated by ultrafiltration under nitrogen to 20 mL as above. Conductivity of the concentrate was adjusted to 40 mS using 6 M guanidine hydrochloride. The concentrate was applied to a column (2 cm × 75 cm) of Sephadex G-75 (1.2 × 5 cm), and the fractions of interest were then applied to a column of (sulfonylethyl)- (SE-) Sephadex C-50 (lot no. 48B-1890-1) equilibrated with 0.6 M guanidine hydrochloride, 0.8 M acetic acid, and 0.001% Triton X-100. As per our experience with the mussel adhesive proteins (Waite, 1983), efficient recovery of proteins from sulfonyl-derivatized dextrans proved highly dependent on batch lot numbers. After a 100-mL wash of the same buffer, the SE-Sephadex column was eluted with a linear gradient up to 1.6 M guanidine hydrochloride. Fractions were directly monitored at 280 nm and assayed for DOPA (Waite, 1983) and by acid/urea polyacrylamide gel electrophoresis in 8 M urea for proteins (see below). Those fractions rich in DOPA were pooled and concentrated by ultrafiltration under nitrogen to a final volume of less than 2 mL and dialyzed against 0.4 M acetic acid. Further purification was achieved by reversed-phase HPLC using a C_8 column (Brownlee RP-300) with an acetonitrile/water gradient where both solvents contain 13 mM trifluoroacetic acid (Waite et al., 1985).

Electrophoresis. Routine electrophoresis was done on polyacrylamide gels (7% acrylamide and 0.2% *N,N'*-methylenebisacrylamide) containing 5% acetic acid with 2 M urea (Panyim & Chalkley, 1969). This system was convenient here because it lends itself readily to staining either for protein with Serva Blue R (Serva Fine Chemicals, Westbury, NY) or for DOPA with the Arnow reagents (Waite, 1983) or the redox cycling method of Paz et al. (1991). Apparent molecular weights were determined by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) using the discontinuous Tris–glycine (Hoefer 1988–1989 Scientific Catalogue, San Francisco, CA, p 131). Isoelectric focusing was performed in the presence of 8 M urea with ampholytes (Servalytes, Serva Fine Chemicals) in the pH range 3–10 according to a procedure suggested by the instruction manual for Hoefer slab gels (Hoefer Scientific Catalogue, p 142).

Preparation of Peptides. Digestion of protein was carried out at a protein concentration of 1.5–2 mg/mL and a protein to enzyme weight ratio of 50:1 in 0.2 M sodium borate (pH 8.2) or 0.1 M Tris–ascorbate (pH 8.2) with 3.5 M urea and 0.1 mM $CaCl_2$ under 40 psi of nitrogen and constant stirring at 22 °C. Trypsin (Boehringer-Mannheim, Indianapolis, IN) digestion was for 12 h, and the progress of the reaction was monitored by removing 10-μL aliquots from the digest at 2-h intervals and applying these to acid/urea gels. The digestion was terminated by addition of 0.3 mL of glacial acetic acid, and the sample was freeze-dried (−80 °C) to a final volume of 1 mL.

Isolation and Characterization of DOPA Peptides. Resolution of the peptides was achieved by reversed-phase (C_8) HPLC using a linear gradient of aqueous acetonitrile (0–20%) with 13 mM trifluoroacetic acid. The sample volume was 1 mL, and the flow rate was 1 mL/min. Fractions absorbing at 280 nm were freeze-dried and resuspended in 50 μL of double glass-distilled water. A 10-μL aliquot was hydrolyzed in 6 N HCl with 5% phenol and 10% trifluoroacetic acid in vacuo at 150 °C for 24, 48, and 72 min to correct for the destruction of Ser, Thr, and DOPA (Tsugita et al., 1987). The amino acid composition of hydrolyzed peptides and proteins was determined following flash evaporation at 60 °C using a single-column Beckman 6300 autoanalyzer. Tryptophan was detected following hydrolysis in 4 N methanesulfonic acid (Simpson et al., 1972). The N-terminal amino acid sequence of Pc-2 and various tryptic peptides was determined by automated Edman degradation using a Porton Instruments 2900 automated microsequencer (Waite, 1991).

Results

The cement glands in the thorax of *P. californica* are known to secrete granules rich in "*o*-diphenols" (Vovelle, 1965). The *o*-diphenols are DOPA-containing proteins accounting for

Table I: Purification of Cement Precursor Proteins

step	volume (mL)	DOPA (mg)	protein (mg)	sp DOPA content[a]	purification (x-fold)	yield
crude extract	10	1.41	108.0	0.013	1	1.00
20% AS ppt	1	1.10	59.0	0.020	1.5	0.85
Sephadex G-75	20	0.91	18.0	0.052	4	0.65
SE-Sephadex	15	0.42	3.6	0.118	9	0.30
HPLC Pc-1	1	0.04	0.23	0.172	14	0.15
HPLC Pc-2	1	0.17	1.37	0.124	10	

[a] The observed specific DOPA content values are strongly dependent on the assays used for DOPA and protein.

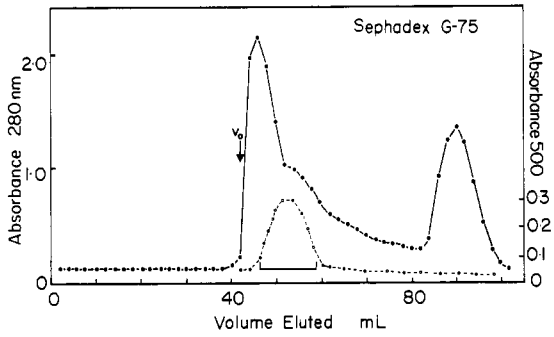

FIGURE 1: Gel filtration chromatography of acetic acid/urea soluble proteins from *P. californica* thoraces. The column is Sephadex G-75 (mesh 20–50 μm) (1.5 cm × 90 cm) eluted with 0.2 M acetic acid and 2 M urea. The flow rate is 0.2 mL/min. Each fraction was monitored for absorbance at 280 nm, and 0.1 mL of every fraction was sacrificed for the assay of DOPA. Fractions included in the bar (designated Pc-2) were pooled for further chromatography.

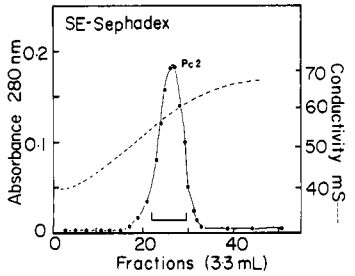

FIGURE 2: Ion-exchange chromatography of cement protein precursor on an SE-Sephadex C-50 minicolumn (5 cm × 1.2 cm) eluted with a linear gradient of guanidine hydrochloride (ranging from 5.5% to 15% w/v) in 5% acetic acid with 0.001% Triton X-100. Fractions (3.3 mL each) were assayed for absorbance at 280 nm and at 500 nm following nitration (not shown). Conductivity (milliSiemens) of every third fraction was measured. A bracket denotes fractions pooled for HPLC.

5–10% of the total extracted protein. As a result, the specific DOPA content of proteins derived from the thorax, expressed in terms of DOPA/protein (wt %), can be used as a measure of purity. The DOPA/protein ratio of purified proteins Pc-1 and Pc-2 is only 15–20 times that of the crude tissue extract (Table I) and thus reflects their relative abundance. Of course, the apparent value of the ratio is somewhat dependent upon the assays selected to detect DOPA and protein. The low yield of the proteins suggests cumulative losses due to adsorption. Two types of DOPA-containing proteins are discernible. Of these, only Pc-2 can be purified to apparent homogeneity at this time, using in succession, gel filtration on Sephadex G-75 (Figure 1), ion exchange on SE-Sephadex C-50 (Figure 2), and reversed-phase C_8 HPLC (Figure 3). The amino acid composition of Pc-2 (Table II) includes high proportions of glycine (30%) and alanine (20%); DOPA and histidine each are present at about 7%, and lysine and tryptophan are present at 6% each. Since Pc-1 is not recoverable following ion-exchange chromatography, this step must be scrupulously

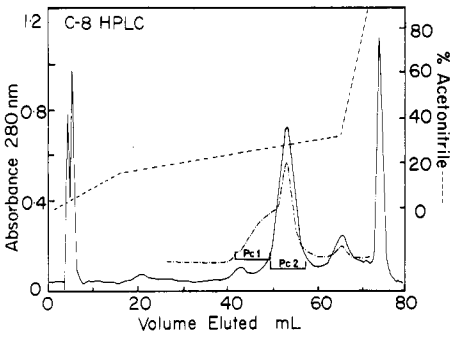

FIGURE 3: Reversed-phase C_8 high-performance liquid chromatography of *P. californica* cement proteins. Of the two elution profiles monitored for absorbance at 280 nm, the broken line represents the sample applied following only gel filtration chromatography on Sephadex G-75, while the solid line was the sample prepared by both gel filtration and subsequent ion-exchange chromatography (SE-Sephadex).

Table II: Comparison of the Amino Acid Composition in Residues per Thousand of the Cement Gland Proteins Pc-1 and Pc-2 with That of Tube Cement of *P. californica*[a]

amino acid	Pc-1	Pc-2	cement[b]
Asx	16	24	20
Thr	11	17	0
Ser	25	37	290
Glx	25	9	9
Pro	17	44	24
Gly	415	296	240
Ala	82	195	65
Cys/2	9	0	0
Val	53	37	44
Met	0	0	3
Ile	11	6	11
Leu	29	32	33
DOPA	98	73	26
Tyr	51	30	33
Phe	4	11	16
His	19	77	28
Lys	124	62	118
Trp	0	31	ND
Arg	11	18	40
total	1000	1000	1000

[a] The confidence limit (95%) of the amino acids is ±5%. [b] Jensen and Morse (1988).

avoided if significant yield of this protein is desired. On the basis of its broad HPLC peak and multiple bands following electrophoresis, Pc-1 more closely resembles a microheterogeneous protein family than a single protein species. It is present as a shoulder of Pc-2 during C_8 HPLC (Figure 3) and distinguished by having higher levels of glycine (42%), lysine (12%), DOPA (10%), and valine (5%) than Pc-2. Alanine is notably lower at 8%, and tryptophan is absent.

Figure 4A illustrates the results of polyacrylamide gel electrophoresis (PAGE) on crude thoracic extracts as well as purified preparations of Pc-1 and Pc-2. Acid/urea PAGE is particularly well-suited for the parallel staining of samples for protein and DOPA using Coomassie Blue R-250 and Arnow's reaction, respectively. Redox cycling of nitro blue tetrazolium was also used to detect the DOPA in Pc-1 and -2 (not shown), but it is not specific for DOPA despite its very high sensitivity (Paz et al., 1991). Pc-1 is resolvable into seven DOPA-containing proteins whereas Pc-2 for all practical purposes appears homogeneous. Pc-2 has an apparent molecular weight of 20 000 (±1000 95% confidence limit) as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (Figure 4B). In contrast, Pc-1 exhibits microheterogeneity and a range of apparent molecular weights (17 000–19 000). The isoelectric point of Pc-2 in the presence

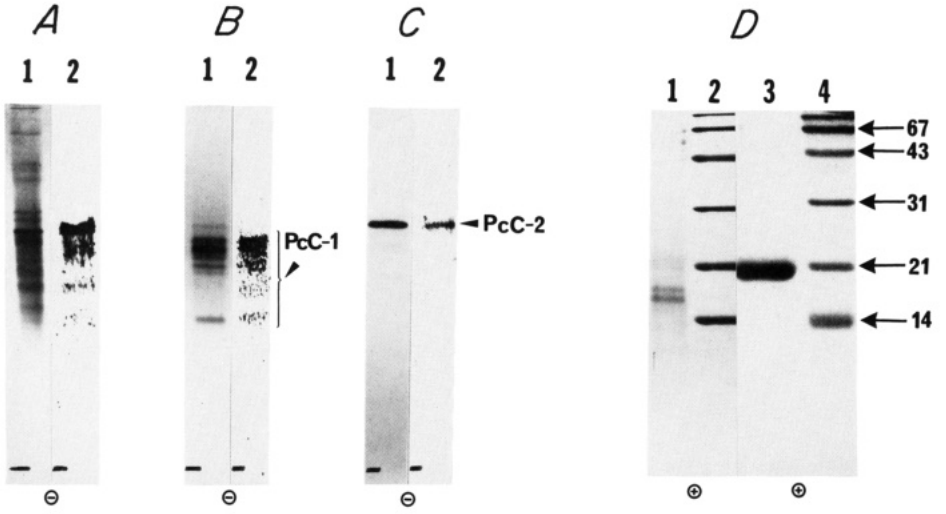

FIGURE 4: Polyacrylamide gel electrophoresis (PAGE) of *P. californica* cement precursors. Panel A: Acid/urea PAGE (7.5% acrylamide) of 30 μg (lane 1) and 60 μg (lane 2) crude acid-extracted thoracic protein. Panel B: 10 μg (lane 1) and 20 μg (lane 2) of Pc-1. Panel C: 12 μg (lane 1) and 25 μg (lane 2) of Pc-2. Lane 1 of each panel was stained with Coomassie Blue R-250, whereas lane 2 was stained for DOPA using Arnow's reagent. Panel D, SDS–PAGE (12.5% acrylamide): 10 μg of Pc-1 (lane 1) and 15 μg of Pc-2 (lane 3) were stained with Serva blue R-250. Lanes 2 and 4 contain the following molecular weight marker proteins: lysozyme (14 000), soybean trypsin inhibitor (21 000), carbonic anhydrase (31 000), ovalbumin (43 000), bovine serum albumin (68 000), and rabbit phosphorylase *b* (91 000).

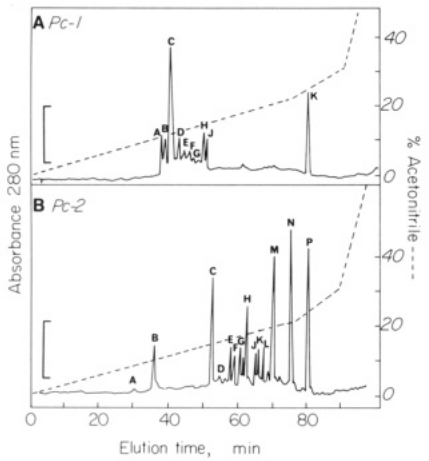

FIGURE 5: Reversed-phase C_8 HPLC of trypsin-digested *P. californica* cement proteins. Panel A: Pc-1. Panel B: Pc-2. The bar at the left denotes an absorbance range of 0.05.

Table III: Primary Sequence of the Amino Terminus of Pc-2

cycle	amino acid	yield (nmol)
1	Ala/Gly/His	0.5/0.4/0.7
2	Gly	1.9
3	Gly	1.6
4	Ala	2.1
5	Gly	1.5
6	Gly	1.7
7	Trp	1.1
8	Ala/Arg	0.6/0.3
9	Gly	1.2
10	Gly	1.3
11	Ser	0.5
12	Ala	0.6
13	Gly	1.1
14	Gly	1.2

of 8 M urea is pH 8.0 ± 0.2; all variants of Pc-1 are more basic and tend to run off the gel at the high end of the pH gradient (pH cutoff ~9.2). Amino-terminal analysis of Pc-2 by gas-phase microsequencing resulted in a glycine-rich sequence of 14 residues (Table III) of which positions 1 (alanine, glycine, and histidine) and 8 (alanine, arginine) are not unique. Because of its obvious electrophoretic heterogeneity, direct sequenator analysis of Pc-1 was not attempted.

PC-1	PEPTIDES	
A	AAGGY*GY*GAK	1.5 μmoles
B	KVGGY*GY*GAK	1.4
C	VGGY*GY*GAK	8.2
D	VGGY*GYGAK	0.7
	VGGYGY*GAK	2.2
E	LGGY*GY*GAK	1.3
F	VGGYGYGAK	1.5
G	IGGYGY*GAK	1.3
H	LGGY*GYGAK	1.4
J	LGGYGYGAK	2.0
	SGIQVR	1.0
K	ALGGY*GAGA	3.0

Pc-2	PEPTIDES	
A	GHPAVHK	0.6 μmoles
	AFSR	1.4
B	AATGY*GY*GS, G, S, K	2.1
	LAHPAVHK	1.3
C	WGHPAVHK	4.0
D	not done	
E	AGGY*GGY*GAHPAV, H, K	1.3
F	AGGAGGWAGGG....K	2.2
G	FGGY*GAHPAVHK	3.0
H	ALGGY*GGY*GAHPAVHK	5.0
I	ALGGY*GGY*GAHP, A, H, V, K	2.3
J	ALGGY*GGY*GAHPAVHAAVHK	3.1
K	not done	
L	AAYNHGFNY*GAN, A, G, S,K	1.7
M	ALGGY*GAGAWGHK	5.0
N	ALGGY*GAGAWGHPAVHK	3.5
P	ALGGY*GAGA	3.0

FIGURE 6: Tryptic peptide sequences of Pc-1 and Pc-2 from *P. californica* thorax. Where composition but not sequence is known, amino acids are separated alphabetically by commas. Y* denotes DOPA.

Trypsin digestion of Pc-1 and Pc-2 was used to prepare peptides for sequencing. Digestion is complete within 12 h at 22 °C, and peptides can be adequately resolved by reversed-phase C_8 HPLC (Figure 5). Gas-phase microsequencing of the peptides suggests that each of the proteins consists largely of distinctive repeated sequence motifs (Figure 6). Due to poor binding of many of the peptides to the sample filter during automated Edman degradation, sequencing to the C-terminal lysine or arginine was not always possible. In such cases, those residues detected by amino acid analysis but not

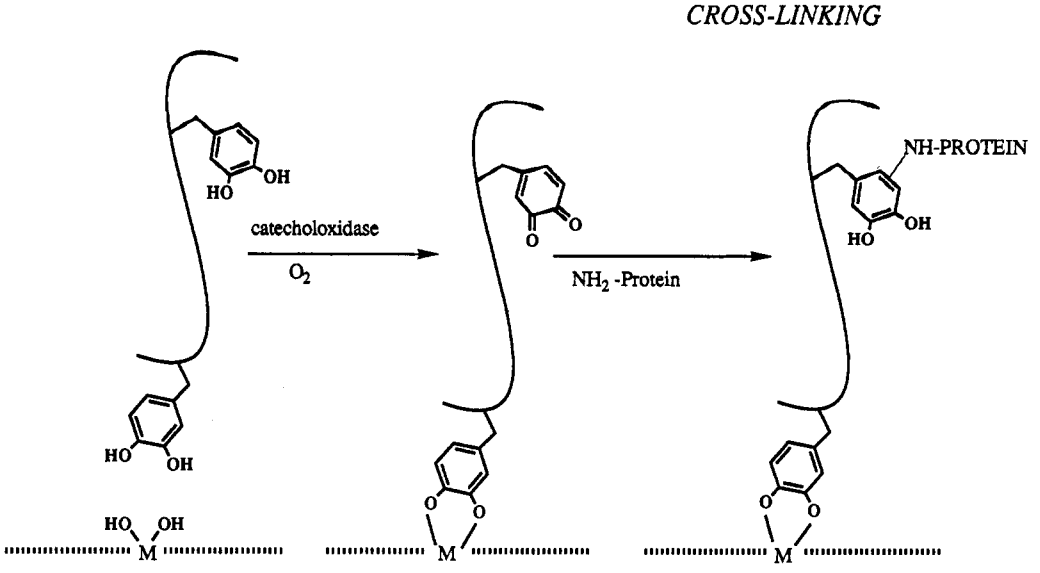

FIGURE 7: The dual cross-linking and surface coupling roles of peptidyl-DOPA in marine adhesive proteins. M represents a metal oxide on the surface of the substratum.

accounted for in the sequence are listed alphabetically and separated by commas after the determined sequence. Much of the primary structure of Pc-2 can be summarized as repeats of the following motif:

$$\{X_1\text{-}[GGY^*]_n\text{-}[GA]_m\text{-}X_2\text{-}[HP(A)V]_p\text{-}HK\}$$

in which X_1 = AL, A, F; X_2 = WG, LA, or nothing; n = 1–2; m = 1–2; and p = 0–2. The peptide ALGGY*GAGA would appear to be at the carboxy terminus of both proteins since the typical C-terminal residue (lysine or arginine) of tryptic peptides is absent. The high apparent concentration of ALGGY*GAGA relative to other peptides in the tryptic digests, however, is anomalous and suggestive of additional complications. Possibilities include poorer chromatographic recovery of other peptides, and protein families that have little more than matching C-termini in common.

In Pc-1, the consensus repeat appears to be quite faithfully represented by $\{X_1$-GGY*GY*GAK$\}$ in which X_1 is mostly V but can also be L, I, AA, and KV. In view of the electrophoretic heterogeneity on acid urea gels, variation in this protein might be largely one of molecular weight.

Discussion

Pc-1 and Pc-2 are the predominant proteins in the thoracic cement glands of *P. californica*, representing close to 10% of the extractable protein. Like the cement, they contain the unusual amino acid DOPA. Unlike the cement, however, which probably contains a blend of proteins, neither protein approaches the 29% serine characteristic of the cement (Jensen & Morse, 1988). This suggests an important role for yet another unidentified serine-rich protein perhaps resembling the sericin of silkworm silk which has up to 33 mol % serine (Komatsu, 1982). Another natural composite that is built underwater, the caddisworm nest (*Stenopsyche griseipennis* McLachlin), has a cement that is 25% glycine, 10% serine, and elevated levels of alanine, valine, tyrosine, histidine, proline, and aspartate but no detectable DOPA (Yamamoto et al., 1988).

Despite our best efforts to purify Pc-1 and Pc-2 to homogeneity, evident heterogeneity persists, particularly in Pc-1. Protein heterogeneity has been correlated with alternative posttranscriptional splicing in collagen (Pihlajaniemi & Tamminen, 1990), silkworm sericin (Michaille et al., 1986) and *Drosophila* chorion precursors (Waring et al., 1990); with multiple gene copies in trematode eggshell proteins (Bobek et al, 1988); and with variable posttranslational hydroxylation efficiencies in the DOPA-containing proteins of *Geukensia demissa* and *Fasciola hepatica* (Waite et al., 1989; Waite & Rice-Ficht, 1987, 1989); however, the underlying mechanism(s) responsible for the heterogeneity of Pc-1 and Pc-2 remains unknown.

The cement precursor proteins of *P. californica* are significant in that they represent the fourth phylum, i.e., Annelida, to possess the amino acid DOPA in the primary sequence of proteins that undergo quinone-tanning. Other phyla are Platyhelminthes (Waite & Rice-Ficht, 1987, 1989), Mollusca (Waite et al., 1985, 1989), and Chordata (Azumi et al., 1990; Dorsett et al., 1987). To wit, the DOPA–protein sequences from various organisms have rather little in common apart from containing DOPA and lysine which are prominent in all but the Ascidian peptides (Azumi et al., 1990; Rzepecki et al., 1991). All but one of the peptides from *P. californica* (Pc-2L) have DOPA in sequences with flanking glycine on both sides. This is reminiscent of the primary sequence of the 17-kDa eggshell precursor of *F. hepatica* (Waite & Rice Ficht, 1987, 1989). Such sequences have also been detected in some marine mussels such as *Aulacomya ater* and *Trichomya hirsuta* the latter of which actually has several repeats of the sequence GY*GAK (Rzepecki et al., 1991). The GAGA repeats of Pc-2 are reminiscent of the crystalline domains of *B. mori* fibroin (Komatsu, 1982). The trytophan- and histidine-containing moiety in some of the Pc-2 repeats is unique among DOPA-containing proteins. Only one other such protein, vitelline protein C of *F. hepatica*, is known to contain both tryptophan and histidine, but in rather different sequences (Waite & Rice-Ficht, 1989).

The distribution of Pc-1 and Pc-2 within the organic mortar is unclear at this time and difficult to determine since quinone-tanning generally conceals the antigenicity of the precursors (Waite, 1990). The byssal holdfast of mussels may

offer the following insight on this point: It is notable that three morphologically distinct zones exist in the pretanned byssal adhesive plaque: (1) the interface (a thin region of continuous contact between the adhesive and the surface of the substratum), (2) bulk adhesive, and (3) an outer coating. Zones 1 and 3 are both enriched in DOPA-containing proteins that appear to be in morphologically different yet immunologically similar structures (Benedict & Waite, 1986). Further ultrastructural, cytochemical, and immunochemical studies will be necessary to determine the distribution of Pc-1 and Pc-2 in the cement of *P. californica*. The possibility that the two proteins are used in the tube lining as well as in a mortar for the masonry cannot be discounted at this time.

The industrial fabrication of environmentally durable adhesive bonds between polymers and inorganic surfaces is a highly complex process which involves a routine number of steps including surface cleaning, the application of surface coupling agents (typically silanes), the removal of excess agent, application of a carefully mixed adhesive formulation (containing monomer, initiator, cross-linker, catalyst, etc.), and setting (Eagland, 1988). Omission or improper execution of any step can lead to adhesive defects and catastrophic failure. The vulnerability of such a heavy reliance on flawless protocol has spurred a move toward a complete overhaul of chemical design. Two recent innovations are noteworthy in this regard: Schmidt and Bell (1988) describe an adhesive for bonding steel which consists of a polymer with pendant functional groups capable of coupling Fe. In the same vein, polymers are now available with functional groups that are capable of cross-linking, e.g. light-activated quinonoid side chains (Cha et al., 1988). The design of polymers with pendant functional groups eliminates, in the former case, the need for the separate application and removal of excess surface coupling agents, and in the latter, the need for thorough mixing of monomer and cross-linker. Thus, both innovations circumvent difficult protocol, and both are anticipated by nature's tube worm cement. Peptidyl-DOPA is a polymer-pendant functional group that can fulfill both surface coupling (McBride & Wesselink, 1988; Baumann, 1963) and cross-linking roles (Davies & Frahn, 1977) (Figure 7). If both of these roles are actually realized, then nature deserves particular recognition for its exquisite economy of design. There is no known synthetic or natural functional group other than peptidyl-DOPA that can effectively serve as both surface coupler and cross-linker. Having said this, we must hasten to add that much remains to be elucidated about sabellariid cement. This includes the identification of the role of His- and Trp-rich primary sequences in surface coupling reactions and the characterization of other key ingredients such as the serine-rich protein(s) and the cross-linking enzyme catecholoxidase (Vovelle, 1965). Sericin, which is the serine- and glycine-rich protein of silkworm silk, may act as a lubricant and shock absorbent (Komatsu, 1982). Such functions would also be well served in the tube cement.

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