Elastomeric gradients: a hedge against stress concentration in marine holdfasts?

J. Herbert Waite*, Eleonora Vaccaro, Chengjun Sun and Jared M. Lucas

Marine Science Institute & Department of Molecular, Cell and Developmental Biology, University of California, Santa Barbara, CA 93106, USA

The byssal threads of marine mussels are elastomeric fibres with a great capacity for absorbing and dissipating energy. Up to 70% of the total absorbed energy can be dissipated in the byssus. Because byssal threads attach the mussel to hard inert surfaces in its habitat, they must combine the need to be good shock absorbers with appropriate matching of Young's modulus between living tissue and a hard substratum such as stone—stiffnesses that can differ by five orders of magnitude. Recent data suggest that improved modulus matching and decreased stress concentration between different portions of the byssus is achieved by the use of protein gradients. Protein gradients in byssal threads are constructed using natural macromolecular chimeras having a central collagenous domain, variable flanking modules and histidine-rich amino and carboxy termini. Stiff silk-like flanking modules prevail distally, while at the animal end, rubbery modules resembling elastin predominate. In between the two thread ends there is a mix of both module types. The histidine-rich termini provide metal binding/cross-linking sites, while collagen domains may confer self-assembly on all parts of the structure. A graded axial distribution of flanking modules is expected to moderate stress concentration in joined materials having disparate moduli.

Keywords: mussel byssus; elastomeric fibres; collagen chimaeras; silk; modulus matching; protein gradient

1. INTRODUCTION

'Without turbulence, there would be no life on earth' (Lugt 1983, p. 120). The full impact of this particular outlook is nowhere more evident than in the high-energy intertidal zone where mussels and other sessile organisms thrive amidst aeration, nutrient cycling and waste removal *ad libitum*. The benefits of a turbulent habitat are considerable, but there are also costs. A major cost for mussels is the production of a robust holdfast or byssus. The byssus provides mussels with the necessary tenacity to survive incessant buffeting by waves. In exchange, a mussel is committed to investing 10% or more of its energy and assimilated nitrogen for making and maintaining a byssus (Jordan & Valiela 1982).

Mussel byssus consists of a bundle of short threads that resemble tiny tendons (figure 1). It is constructed entirely of non-cellular, extracellular matrix. In *Mytilus* species (*M. edulis* and *M. galloprovincialis*), each new thread has dimensions of a few centimetres in length and less than 0.1 mm in diameter and is produced in *ca.* 5 min, in the ventral groove of the foot, by a process akin to reaction injection moulding (Waite 1992). Like tendons, the threads originate from the byssal retractor muscles by way of a rooted stem; unlike tendons, byssal threads do not 'insert' into foreign objects as tendons do to bone (Currey 1984). Instead, byssal adhesive plaques bond only to the surface of exogenous substrata such as stone (Tamarin *et al.* 1976). For morphological clarity, the byssus is divided

into four parts (from proximal to distal): root, stem, thread and plaque or pad. Each thread is further divided into proximal and distal portions according to appearance, i.e. smooth and stiff for the distal, soft and crimped for the proximal. The formation and ultrastructure of mussel byssus has been described in detail elsewhere (Bairati 1991; Waite 1992).

2. SHOCK-ABSORBING TETHERS

Based on the generally accepted features of elastomers (Gibson & Ashby 1997), byssal threads are ostensibly elastomeric: Young's modulus is low (the range is 10–500 MPa), extensibility can be as high as 200% and there is restorative recoil. In common with other protein elastomers such as elastin, resilin and abductin, byssal threads are quite tough (they exhibit high strain energy at break) (table 1). However, they are better than most at energy dissipation. Toughness and energy dissipation are both crucial properties for holdfasts. Energy dissipation in fibres subjected to cyclic stress–strain analysis is frequently normalized with respect to the total absorbed energy and reported as hysteresis or percentage hysteresis (table 1). Hysteresis reflects a shock-damping capacity and is caricatured for one byssal thread by a confined spring and piston analogue (figure 1*a*). The water flow associated with a wave, for example, can deflect a typical sessile mussel by lift and drag forces. By restraining the mussel's deflection, a byssal thread assumes the load and stretches (A→B, upper curve of the grey shaded area). As the wave passes, the load is relieved and the thread relaxes (B→A, lower curve). Instant recoil is prevented by energy dissipation

* Author for correspondence (waite@lifesci.ucsb.edu).

Figure 1. Stress-strain cycles of byssus from *Mytilus galloprovincialis*. One thread is modelled as a spring-loaded shock absorber showing hysteresis and breakdown into distal and proximal contributions. Hysteresis is about 72% for (a) the whole thread and (b) distal portion, and slightly lower at 62% for (c) the proximal portion. All samples were extended to 0.5 (strain rate $0.4\text{--}0.7\text{ s}^{-1}$). (d) A mussel with a typical byssal attachment and extended foot. An enlarged dissected byssus is shown with all but one thread removed for clarity. Thread diameter: $50\text{--}100\text{ }\mu\text{m}$; length: $2\text{--}4\text{ cm}$. Thread number per mussel in the field has a typical range of $20\text{--}200$.

(viscous effects) and represented by friction in the piston element, thus sparing the mussel from being dashed against the rocks. The stress-strain cycle for one thread has been dissected into separate mechanical contributions for the distal and proximal portions of the thread (Smeathers & Vincent 1979; Price 1981; Bell & Gosline 1996) (figure 1b). Of these, the distal portion is stronger, stiffer and superior at damping (70% hysteresis), while the proximal portion is softer and weaker, with a lower, but still significant, hysteresis at 40%. Other protein structures show hysteresis ranging from 10% in elastin and tendon to a high of over 80% for titin-derived passive tension in skeletal muscle (Wang *et al.* 1993). MA and viscid spider silks both have a hysteresis of 65% that is appropriate given their role in prey capture (Gosline *et al.* 1999).

Figure 2. (a) Stress-strain cycling of byssal threads for two cycles ('waves') to 0.7 strain. (b) Stress softening is shown for thread I in the 2nd cycle leading to recruitment of thread II. (c) Modulus matching in proximal and distal portions over five consecutive cycles appears to involve stress softening and strain stiffening in distal and proximal portions, respectively.

The mechanical properties of byssal threads are further complicated by time- and strain-dependent behaviour. Bell (as cited in Waite *et al.* 1998) demonstrated that, when strained beyond its yield point, the distal portion exhibited a dramatic stress softening, i.e. the initial modulus of the second cycle was reduced to about 20% of the modulus in the first cycle. Hysteresis was reduced as well; complete recovery to the modulus of the first cycle was slow e.g. longer than 24 h, but significant partial recovery can occur within 1 h (Bell 1998). Ironically, stress softening in individual threads may enhance the toughness in the entire byssus because it redistributes recurring loads. As illustrated in figure 2, a roughly radial distribution of threads by the mussel subjected to uniaxial loading results in stress concentration at the leading thread (#I). Suppose that thread #I has been extended beyond its yield point by the first wave. Enter a second wave from the same direction. Due to stress softening, thread #I's modulus has decreased from 500 to 80 MPa. Consequently, it extends with little resistance to loading. By so doing, threads #II and III, which still have high initial stiffness, are recruited into action. Presumably, the process of stress softening followed by new recruitment continues until as many threads

Table 1. Relative magnitude of tensile properties for structural proteins relevant to the byssus of *M. galloprovincialis*. (Strength or $\sigma_{\max}$ is the force to break divided by the cross-sectional area, extensibility or $\epsilon_{\max}$ is the change in length (from initial to breaking) divided by the initial length, stiffness (E_i) is usually taken as the initial slope of the σ versus ϵ curve, and the area under the σ versus ϵ curve gives the breaking strain energy or toughness. Hysteresis (%) is determined from cyclic stress-strain at non-breaking strains and represents the ratio of energy dissipated to the total energy absorbed. nd, not determined.)

tensile element	stiffness E_i (GPa)	$\sigma_{\max}$ GPa	$\epsilon_{\max}$	hysteresis (%)	toughness (MJ m^{-3})	source
elastin	0.001	0.002	1.5	10	7.5	Gosline <i>et al.</i> (1999).
periostracum	0.10	nd	0.9	nd	nd	Authors' observations.
tendon collagen	1.5	0.15	0.12	7	5	Gosline <i>et al.</i> (1999).
keratin, wool	0.5	0.2	0.5	45	20	Wainwright <i>et al.</i> (1976).
silk, viscid	0.003	0.5	2.7	65	150	Gosline <i>et al.</i> (1999).
silk, MA	10	1.1	0.3	65	160	Gosline <i>et al.</i> (1999).
titin (muscle)	0.002	nd	2	81	nd	Wang <i>et al.</i> (1993).
byssus, distal	0.50	0.15	0.8	72	50	Authors' observations.
proximal	0.05	0.02	1.6	47	15

Figure 3. (a) Incremental steps in stiffness E_{init} in byssal attachment to stone. The initial moduli for different portions were determined from byssal sections taken from *M. galloprovincialis*. The strain rate was between 0.4 and 0.7 s^{-1} . (b) Stresses arising between two materials A and B with different moduli in a butt joint. Both A and B are assumed to have the same Poisson ratio (0.4) and the axial stress σ_z is assumed to be equal to 1 . Residual stresses, σ_r and σ_θ , denote the radial and circumferential stress, respectively, and are assumed to be equal. The problem looks at $E_B \leq E_A$.

as possible contribute to load sharing. The proximal portion also shows a tendency to change stiffness with cyclic loading. In this case, there is strain stiffening from an initial modulus of 35 MPa to an asymptotic leveling at 50 MPa (figure 2b), an increase of about 40% .

3. INCREMENTAL MODULUS

In mediating the attachment of a mussel to stone, the byssus depends on a chain of morphological entities, each with mechanical attributes that are well separated from its immediate neighbours. This is illustrated with respect to stiffness, for example (figure 3a). The modulus of skeletal muscle under passive tension is estimated at 0.2 MPa (Wang *et al.* 1993). The stem has a modulus of *ca.* 10 MPa (E. Vaccaro, unpublished data). Distinct proximal and distal portions within each thread differ in modulus by an order of magnitude, e.g. 50 MPa (proximal) and 500 MPa (distal) in *M. galloprovincialis*. Finally, each thread is attached to a rock or shell with a modulus of

$25\,000 \text{ MPa}$ or more. Although the moduli appear to increase incrementally from the animal's soft tissues to the rock, many of these steps are nonetheless abrupt leaps in stiffness.

In manufactured materials, leaps in mechanical properties usually reflect structural discontinuities and raise serious engineering concerns. When joining man-made materials, it is good design practice to ensure that critical material properties are well matched. Depending on the application, critical properties might include, among others, Young's modulus, coefficient of expansion and conductivity. The modulus is the most salient in considering the byssus. Without a good match in modulus, joined materials deform to different extents during loading. Concentrated shear and radial stresses, called residual stresses, are generated where the interface between two mismatched materials intersects a free edge. These can lead to crack propagation parallel to the interface as well as other modes of failure (Rabin *et al.* 1995). An interesting treatment of residual stresses in a model butt joint is illus-

Table 2. Compositional analysis of *M. edulis* byssal threads. Water content was determined by weighing freshly collected blotted threads before and after freeze-drying. Ash content was estimated from the weight remaining after combustion at 530 °C. The proportion of collagen in threads was estimated from the 4-hydroxyproline content in proximal and distal portions divided by the 4-hydroxyproline content of purified Col-P and -D. Composition values are given in % $\pm$ s.d. when known. *n*, number of observations.

byssal thread analysis	%	<i>n</i>	method
water content (% total wt)	70 $\pm$ 3	8	microbalance ^a
dry weight (% total wt)	30 $\pm$ 2	8	lyophilization ^a
ash (% dry wt)	4.8	1	combustion ^a
Cu/Fe/Zn (% dry wt)	0.1	—	atomic absorption ^c
other cations (% dry wt)	0.2	3	atomic absorption ^c
hexoses (% dry wt)	2.5	3	anthrone ^b
protein (% dry wt)	95 $\pm$ 3	5	amino-acid analysis ^{b,d}
collagen (% protein)			
distal	48 $\pm$ 2	4	hydroxyproline ratio ^{c,d}
proximal	33 $\pm$ 4	4	hydroxyproline ratio ^{c,d}
preCol (% protein)			
distal	96		Col $\equiv$ preC ol/2 ^c
proximal	66		Col $\equiv$ preCol/2 ^c
other protein, proximal	34		—

^a C. Sun (unpublished data); ^b Pikkarainen *et al.* (1968); ^c Coombs & Keller (1981); ^d Mascolo & Waite (1986); ^e Qin & Waite (1995).

trated in figure 3*b* (Kinloch 1987). Here, two cylindrical materials, A and B, bonded at a common interface, are subjected to uniaxial loading. When the two materials have identical Young's moduli (E_B , E_A) and Poisson ratios (ν_B , ν_A), then the axial stress, σ_z , is the only stress in the system. However, when the materials are dissimilar, residual radial and circumferential stresses, σ_θ , σ_r , are generated where the interface meets a free edge according to equation (3.1):

$$\sigma_\theta = \sigma_r = [\nu_B - E_B \nu_A / E_A][\sigma_z / (1 - \nu_B)]. \quad (3.1)$$

The example in figure 3*b* assumes that $\sigma_z = 1.0$ and that A and B have the same Poisson ratio (0.4) over a range of E_B/E_A . Note that σ_θ and/or σ_r can approach two thirds of σ_z as E_B decreases relative to E_A (Kinloch 1987). When B has a stiffness that is one tenth that of A, such as the difference between the proximal and distal portions of thread, the expected σ_r is nearly three-fifths that of σ_z .

In byssus, are the stepwise moduli correlated with discontinuities in the structure? How are the residual stresses caused by modulus mismatch avoided? The joints between the attachment plaque and stone and, above that, between the plaque and the distal thread do indeed exhibit abrupt structural discontinuities (Tamarin *et al.* 1976; Benedict & Waite 1986). These are counterbalanced somewhat by large interfacial areas. Another discontinuity is apparent at the junction between the stem-root and retractor muscles (Tamarin 1975). The 'joint' between the distal and proximal portions of the thread has not been systematically scrutinized by transmission electron microscopy. This is surprising given the obvious morphological differences between the two, e.g. different diameters and smooth or corrugated surfaces on proximal and distal portions, respectively, evident upon examination by light or SEM (Bairati 1991); personal observations). The proximal and distal portions of a mytilid thread, but not the area joining them, have been subjected to more detailed scrutiny by TEM than any other byssal structure (Vitellaro-Zuccarello

et al. 1983; Benedict & Waite 1986; Bairati 1991). The distal portion consists of densely fused fibrils (diameter 7–8 nm) all orientated parallel to the thread axis and dispersed with occasional voids. In transverse sections, the fibrils appear to be in hexagonal and pentagonal patterns with 20 nm centre-to-centre distances (Bairati & Vitellaro-Zuccarello 1976). The proximal portion, in contrast, has some densely packed fibrils near the thread cuticle; solitary undulating fibrils (diameter 7–8 nm), or small clusters thereof, dominate the rest of the core. The undulations have an average pitch and diameter of *ca.* 70 and 100 nm, respectively (Bairati & Vitellaro-Zuccarello 1976; Vitellaro-Zuccarello *et al.* 1983).

While TEM has yet to be fully exploited to investigate the distal to proximal transition, a variety of other analytical techniques, including fibre X-ray diffraction (Rudall 1955), light birefringence (Mercer 1952; Gathercole & Keller 1975; Bairati & Vitellaro-Zuccarello 1974), amino-acid composition (Mascolo & Waite 1986) and protein extraction (Qin & Waite 1995) have detected the presence of gradients in byssal threads. Before embarking on the subject of gradients, however, the proteins in byssal threads deserve an introduction.

4. PROTEINS IN BYSSAL THREADS

Freshly collected byssal threads are about 70% water, by weight. Of the dry weight, about 96% is present as protein with carbohydrates and inorganic residue (ash) accounts for the rest (table 2). Ash content is quite variable depending on the extent of imbibed seawater removal. It consists of salts, including heavy and transition metals (Coombs & Keller 1981). Collagen represents about 48% of the protein composition of the distal and 33% of the proximal, portion. This agrees with an independent estimate of $\leq 50\%$ collagen based on fibre X-ray diffraction of the distal portion (Rudall 1955). Non-

collagenous proteins account for a significant part of the proximal portion of the thread (*ca.* 34%).

Isolation of proteins from mytilid byssus is greatly hampered by the extremely tough and insoluble properties of the structure. In general, proteins are more extractable from younger threads than older threads; they are also more extractable from the proximal than distal portions. Perhaps, in the latter case, this is due to the smaller proportion of matrix in distal byssus. A higher cross-link density is also possible. To isolate byssal proteins, we have used two extractive strategies, one for collagens (A) and the other for non-cross-linked proteins (B). Strategy A is the standard pepsinization method whereby byssus is incubated for many hours at 5–10 °C with pepsin at pH 3–5 (Qin & Waite 1995). As native triple-helical collagen is largely pepsin-resistant, this treatment releases soluble collagen domains (Col-P, Col-NG and Col-D) and many smaller peptide fragments. Strategy B involves the use of 8 M urea buffered at pH 3–4. This treatment releases most, or all, of the proteins not covalently cross-linked in the structure (Benedict & Waite 1986).

The collagen domains released by strategy A appear to be homotrimers with α -chain monomer masses of 40–50 kDa. The α -chains have been characterized by partial peptide mapping and sequencing (Qin & Waite 1995). Edman sequence data were used to make degenerate oligonucleotides that allowed preferential PCR amplification of a mussel foot cDNA library, and later, selection and amplification of cDNAs corresponding to complete α -chain sequences of the byssal collagens (Coyne *et al.* 1997; Qin *et al.* 1997). These collagens are referred to as 'pre-pepsinized collagens' or preCols (Qin & Waite 1995; Waite *et al.* 1998) and have been described as naturally occurring molecular 'chimaeras' on account of their linear array of structural motifs (figure 4): (i) a central, kinked collagen domain representing 52–58% of the sequence; (ii) an acidic patch (C-side of collagen); (iii) variable flanking domains (both N- and C-side), and (iv) amino- and carboxy-termini rich in histidine and 3, 4-DOPA residues. The variable flanking domains in preCol D have sequence motifs reminiscent of spider dragline and *Antheraea* cocoon silk (Guerette *et al.* 1996; Sezutsu & Yukuhiro 2000). As demonstrated in table 3, there are six slightly substituted poly-alanine blocks that, in spun silks, give rise to crystalline β -sheets (Simmons *et al.* 1996). Pre-Col NG has Gly-rich sequences (66% Gly) (Qin & Waite 1998) for which several homologues are worth noting: mussel periostracum is *ca.* 55% Gly (Waite *et al.* 1979), as are certain plant cell-wall proteins (Sachetto-Martins *et al.* 2000) and, of course, the amorphous regions of spider silk (Lewis 1992; Guerette *et al.* 1996). PreCol-P-flanking sequences resemble the proline-containing pentapeptides of elastin (Brown-Augsburger *et al.* 1995) and flagelliform (viscid) silks (Hayashi & Lewis 1998) (table 3). Complete sequences for α -chains of preCol-D, NG and P are available in GenBank and have been discussed in a previous review (Waite *et al.* 1998). Using specific polyclonal antibodies and colloidal gold immunohistochemistry, preCol-P was shown to be associated with the undulating fibrils in the core of the proximal portion, while preCol-D was localized to the densely-packed fibril bundles in the distal portion (Qin & Waite 1995). Specific antibodies for preCol-NG are not available.

Figure 4. (a) Schematic block structure of tropocollagen and the preCols P, D and NG. The collagenous domain of type I sea urchin collagen (Exposito *et al.* 1992) represents more than 95% of the tropocollagen sequence while the non-collagenous sequence is 5% (shown in scales). (b) In contrast, a central collagen occupies about 50% of the composition of each preCol. (c(i)) The closest homology of the flanking domains in each preCol is listed and sequence motifs are given in table 3. (c(ii)) Central collagen occupies about 50% of the composition of each preCol. The effect of missing amino acids in each collagen domain is modelled using SYBIL (v. 6.7.1, Tripos Inc., St Louis, MO, USA; King *et al.* 1996).

Strategy B liberates a variety of proteins, particularly from proximal byssus. Notable among these is PTMP-1, which was recently purified, characterized and cloned from two *Mytilus* species (C. Sun & J. M. Lucas, unpublished data). The PTMP-1 mass, as determined by matrix-assisted laser desorption/ionisation with time of flight mass spectrometry is 50 kDa compared with the calculated 47.5 kDa based cDNA-deduced primary sequence. Carbohydrates probably account for most of the difference, as PTMP is a glycoprotein. The structure of PTMP can be modelled by its strong homology to known proteins in the database (SwissProt). More than 80% of the primary sequence is present as two A-type domains of von Willebrand factor (also known as I-type integrin domains) (Pareti *et al.* 1987) (figure 5a). Each of these domains contains a metal-assisted collagen-binding sequence: DXSSS. Recent X-ray crystallographic analysis of the complex formed by the I-domain of integrin, Mg and a collagenous peptide revealed that an aspartate, two waters and two serines octahedrally coordinate Mg^{2+} in the DXSSS sequence, and by a glutamate residue from the collagen (Bella & Berman 2000).

5. EMERGENCE OF PROTEIN GRADIENTS

Mascolo & Waite (1986) first identified biochemical gradients in byssal threads of *Mytilus*. After serially cutting

Table 3. Some sequence motifs for flanking domains in three byssal preCols from *M. edulis* and in related proteins. Number of repeats in parentheses.

protein	pentapeptide	glycine clusters	poly-A runs
preCol-P ^a <i>M. edulis</i>	GXGPG (6) GGGPG (5) XGGPG (4) GGXPG (2) X = I, F, A	ZGG (39) Z = I, F, V, A	ASAZAAAZAN (3) Z = N, R
elastin ^b <i>Bos</i>	XGXPG (17) GXXPG (8) GGXPG (4) GXGPG (3) X = V, F, A, G	ZGG (20) Z = L, I, V, A	AAAKAAAKAA (10)
viscid silk ^c <i>Nephila</i>	GXGPG (235) X = A, S, Y, V	GGZ (28) Z = A, S, T	none
preCol-D ^d <i>M. edulis</i>	GAGPG (2)	ZGG(G) (40) Z = L, A, F	AAAZAAA(A)ZAAA (6) Z = R, N, S
spidroin1, MA silk ^e <i>Nephila</i>		ZGG(G) (100)	AAAAAAAAAAAA (25)
wild silk ^f <i>Antherea</i>	none	GGY (117) GGZ (51) Z = S, A, V	AAAAAAAAAAAAA (80)
preCol-NG ^g <i>M. edulis</i>	none	ZGG(G) (65) Z = L, A, N, H	ASAAARAAAAA (2)
cell wall protein ^h <i>Petunia</i>	none	ZGGG(G) _n (50) Z = A, V, L	none

^a Coyne *et al.* (1997); ^b Brown-Augsburger *et al.* (1995); ^c Hayashi & Lewis (1998); ^d Qin *et al.* (1997); ^e Lewis (1992); ^f Sezutsu & Yukuhiro (2000); ^g Qin & Waite (1998); ^h Sachetto-Martins *et al.* (2000).

byssal threads into 2 mm segments, Mascolo then hydrolysed these and determined their amino-acid compositions. Glycine showed the greatest change, from a high of 330 residues per 1000 at the far-distal end to 240 residues per 1000 at the far-proximal end; 4-hydroxyproline showed a similar trend, though the change was smaller. Taken together, the Gly and Hyp gradients suggested collagens. Qin & Waite (1995) identified gradient-specific collagens by isolating two pepsin-resistant collagen fragments, Col-P and Col-D, from serial byssal thread sections and demonstrating that the distribution of these along the thread axis was graded. Graded distributions of two preCols (D and P) were also evident from Western blots of protein extracted from successive serial sections of byssal threads and mussel feet using specific polyclonal antibodies for the collagenous domains of preCol-D and P (Qin & Waite 1995), as well as *in situ* hybridization using preCol-specific probes (Qin *et al.* 1997; Qin & Waite 1998). Because of the inevitable use of four to six tissue sections for discontinuous analysis, these results do not specify the precise shape but rather the general trend of the gradients. The distribution of preCol-D tapers off in a distal-to-proximal direction (figure 6a), while preCol-P decreases in a proximal-to-distal direction reaching extinction before mid-thread in *M. edulis*. PreCol-NG ('no gradient'), in contrast, is uniformly distributed. Evidently, these gradients are produced prior to secretion because they are already detectable in expression libraries prepared from serial foot sections (Qin & Waite 1998). Here, the relative abundance of each preCol mRNA can be precisely titrated using the rRNA EukA gene product as internal reference (Qin *et al.* 1997; Qin & Waite 1998). Another gradient involving two variants of preCol-P has recently

been reported to be present where the proximal thread fuses with the stem (Coyne & Waite 2000).

6. ASSEMBLY AND CROSS-LINKING

There are several distinct but interdependent determinants of protein fibre assembly: (i) covalent interactions between tensile elements, (ii) axial or linear sequence, (iii) register, and (iv) packing density. Sufficient basis exists for some insight into the covalent interactions between preCols. These are summarized in figure 7. Given the enrichment of histidine and DOPA in the amino- and carboxy-terminal sequences of all preCols, Waite *et al.* (1998) proposed that these residues mediate end-to-end preCol binding as chelate complexes. Although chelate complexes are expected to provide axial interactions in isolated preCol polymer chains, they could also be lateral at higher packing densities. The primary metals involved are Zn, Cu, and Fe: these three are detected in byssus at up to 0.1% of dry weight (table 2). If this amount were chelated by preCols it would represent 3–4 metals per preCol in the distal portion. Many other metals are present at trace levels (Koide *et al.* 1982; Coombs & Keller 1981). The stability constants of chelates formed between Fe(III) and DOPA-containing byssal precursors at pH 7.5 and 20 °C were calculated at over 10⁴⁰ (Taylor *et al.* 1996). Histidine in glycine-rich sequences, e.g. Gly-His-Gly, binds Cu(II) at 10²⁰ (Smith *et al.* 1987); this tripeptide alone is repeated four times in the N-terminus of preCol-NG, and once or twice in the C-termini of -P and -D (Waite *et al.* 1998). The role of metal chelates in the cohesive assembly of preCols is also suggested by two recent observations: preCols were precipitated by

Figure 5. (a) PTMP structure showing the prominence of two von Willebrand factor domains. (b) The demonstrated tendency of these domains to bind collagen under shear (Smith *et al.* 2000) is modelled to suggest matrix-fibre interactions in byssus during shear stiffening; (i) without shear, (ii) with shear.

Figure 6. (a) Gradients of chimaeric macromolecules preCol-D, -NG and -P in byssal threads from *M. edulis*. Gradient shape is approximate, as are relative proportions. (b) Model 1 of axial and lateral assembly of preCol dimers (poly [-NG-X]) in mid-byssal threads where the gradient is thought to occur. All elements are assembled N→C with full lateral register. (c) Model 2 of homopolymer axial assembly involving poly[-D] and poly[-NG]. PreCols are successively N-to-N and C-to-C. The interfibrillar pattern denotes matrix protein. Arrow tails are N-terminal and heads are C-terminal.

micromolar amounts of Cu^{2+} (E. Vaccaro, unpublished data), and EDTA treatment abolished the yield point in distal byssal threads (Vaccaro & Waite 2001).

End-to-end stabilization of preCols is also re-enforceable by aryl coupling (Y-Y in figure 7a). DOPA residues not engaged in chelate complexes are prone to oxidation in seawater. In a study of byssus 'stressed' by conditions of high flow and aeration, the primary product of oxidation was found by solid-state NMR with ^2H - and ^{13}C -tyrosine-labelled byssus to be 5,5' diDOPA (McDowell *et al.* 1999). diDOPA was also formed during *in vitro* oxidation of DOPA-containing byssal precursor proteins (Burzio & Waite 2000). Another type of covalent interaction is the disulphide bond (-S-S- in figure 7a). As indicated above, preCols are trimers. Each α -chain of preCol-

D and -NG contains a single cysteine residue located in the latter half of the collagen domain (Qin & Waite 1998; Qin *et al.* 1997). PreCol-P has no cysteine. Disulphides form spontaneously from proximate pairs of cysteine residues by one-electron oxidation. Disulphide formation between neighbouring preCols, or preCol and a matrix protein, would represent lateral interactions. PreCol-D and -NG have a maximum capacity of three lateral disulphide interactions per molecule and a minimum of one (if the other two are involved in an intramolecular disulphide). Cross-links such as aldimines or aldol condensations typical of tendon collagen have not been detected in byssus (Van Ness *et al.* 1988).

The nature of axial and lateral order is at the root of understanding how the graded distribution of preCol-D and -P translates into a functional mechanical gradient. Concepts from polymer science bring to mind two paradigms of axial or linear sequence: homopolymers (those constructed from a single kind of repeat unit e.g. poly[D] in which bracketed letters such as D refer to a particular preCol) and copolymers, with two or more types of ordered or random repeat units (e.g. poly[NG-P] or poly[$\text{D}_x\text{-NG}_y\text{-P}_z$], where subscripts denote relative proportions of each in the chain). Plausible models reconciling axial sequence with the stochastic distribution of preCols in byssal threads can be contrived for almost any combination of homo- and/or copolymer chains in which spatial variability is controllable. Given the metal-induced 'stickiness' of the histidine-rich ends, even short chains would readily combine into longer ones upon the addition of Cu and/or Zn (see, for example, Lilius *et al.* 1991).

Two slightly different models of axial assembly will be examined. The axial model (model 1) proposed by Qin & Waite (1998) is based on the dimer NG-X (X is D or P) as the fundamental repeating unit. It assumes that preCols, like most collagens, assemble in an N→C orientation and that this assembly requires the coupling of specific sequences in the His-rich domains of adjacent preCols. Several such 'hand-shaking signatures' are proposed: (i) preCol-D and -P have the tyrosine and/or DOPA-containing sequences, DY and KPGY, in their N-

Figure 7. Models of preCol assembly in *Mytilus* byssus. (a) Summary of cross-links implicated in assembled preCols. diDopa cross-links have been detected by solid-state NMR (see § 6); disulphides are present in isolated preCols. Metal chelates are suggested by protein sequence and by the effect of EDTA on the mechanical properties (Vaccaro & Waite 2001). (b) View of tightly packed and fully aligned preCols in the distal portion in the equal stress or Reuss estimate of model 1. Loading is normal to the plane of each layer. Moduli and volume fractions for the collagen, silk-like and Gly-rich domains are taken as 1.5, 10 and 0.1 GPa, and 0.5, 0.25 and 0.25 GPa, respectively. (c) Voigt estimate of model 2. Volume fractions and moduli are the same as above.

and C-termini, respectively (Waite *et al.* 1998). The reverse pattern holds for preCol-NG. (ii) PreCol-D and -P both contain HAXSAG and HAHAAH near the N-terminus and HGG (1-2xs) at the C-terminus. Again, the reverse pattern is true in preCol-NG. Coupling preCols by way of such sequences may seem contrived, but it is reasonable here given that the local environment of histidine and DOPA functionalities has a pronounced effect on metal chelation and/or cross-linking ability (Smith *et al.* 1987; Burzio & Waite 2000). Glycine and alanine are common spacers for histidine residues involved in metal chelation, but their effects on the affinity and specificity of histidine towards metals such as Zn, Ni and Cu, for example, are different (Smith *et al.* 1987). What emerges from this model is a copolymer of two specific dimers (poly[NG-D] and poly[NG-P]), in which the proportion of the two can be gradually changed. Thus, beginning at the distal end, preCol-NG would be required as a linker between the C-terminus of one preCol-D and the N-terminus of the following preCol-D, and, eventually, preCol-P (figure 6b).

Another interesting model (model 2) addressing the distributions of preCol-D, -NG and -P in byssal threads involves spatially variable homopolymers. With this one, we discard the assumption that axial orientation must necessarily be head-to-tail. Both head-to-head and tail-to-tail orientations were observed in the kinked collagens of the dogfish EC by rotary shadowing (Knupp *et al.* 1999). Head-to-head interactions would allow a linear sequence as pictured in figure 6c with preCols in N-to-N-terminal and C-to-C-terminal couplings. Note that this still accommodates 'hand-shaking' signatures. PreCol-NG would form uniform homopolymers (poly[-NG]) from the distal to proximal end of the thread, while the poly[-D] chains formed distally would show an increasing tendency to substitution by preCol-P as they pass from thread mid-length into the proximal portion (figure 6c).

'Register' refers to the nature of alignment of a linear

tensile element with its lateral neighbours. In models of fibre-forming interstitial type I collagens, axial assembly such as that described above is prevented by a gap region about 36 nm wide separating each collagen molecule from its linear neighbours. A consequence of this gap is that fibre formation (also known as fibrillogenesis) in collagens is driven by lateral rather than axial interactions (Prockop & Fertala 1998). Lateral interactions between neighbouring tropocollagens are uniformly shifted by a quarter-length, or about 75 nm (Prockop *et al.* 1979). Quarter-stagger periodicity is not observed in byssus and is difficult to envision in byssal preCols for the following reasons: (i) collagen domains cover only 50% of the pre-Col molecule (compared with 95% in tropocollagens), (ii) preCol collagen domains are markedly kinked by one or more missing glycine residues, and (iii) the histidine-rich termini are large, extended secondary structures. Predicted metal chelation between the histidine-rich N- and C-terminal domains would prevent gaps between preCols such as those present in type I collagen. We suspect that densely packed distal preCols are more likely to be in register than the loosely packed ones of the proximal portion: unique kinks, stacking of β -sheet crystals (see fig. 4 in Waite *et al.* 1998) and the formation of lateral disulphides would all favour full register between packed poly[D] segments.

7. MECHANICAL MODELS

Perhaps future work will reveal new facts that disqualify these models. Models are meant to challenge the data, not to validate them. Models 1 and 2 attempt to reconcile preCol assembly with the intricacies of available sequence, domain structure, preferred orientation of collagens and metal-binding environments, as well as the stochastic pre-Col distributions. But are the models consistent with the known mechanical properties of byssal threads? The answer is affirmative if certain assumptions are made, the

main one being that the stiffness of the silk-like (D), elastin-like (P) and glycine-rich flanking domains can be based on the stiffness of spider MA silk, elastin and periostracum, respectively (table 1). The preCol collagen domain is also presumed to be like tendon collagen, although the unknown consequence of missing Gly residues must add some uncertainty to this.

Mechanical models have been designed to assess the effect of serial or parallel components in composite materials (Harris 1980). Of the two portions, the distal is better disposed to modelling since, by composition, 96% of it comprises densely fused chains of collagen, silk and Gly-rich segments coupled with disulphides. If neighbouring silk segments are in tight register, perhaps with stacked β -sheets (figure 7b), then the array would resemble a smectic liquid crystal and conform well to the requirements for a series of alternating hard and soft layers under normal uniaxial tension (Model 1). The Reuss estimate (equation (7.1)) for calculating a composite modulus for such a structure is

$$1/E_c = V_1/E_1 + V_2/E_2 + \text{etc.} \quad (7.1)$$

Using volume fractions (V_n) of 0.5 for collagen and 0.25 for silk- and periostracum-like domains and moduli (E_n) of 1.5, 10 and 0.1 GPa for collagen, silk and periostracum, respectively, a composite E_c of 350 MPa is calculated. This is well within the range of measured moduli for the distal portion of byssus from *Mytilus* species for which we have a byssal protein sequence (range 150–500 MPa: Price (1981); Bell & Gosline (1996); Smeathers & Vincent (1979); Vaccaro & Waite (2001)). If one segregates the preCol-D and -NG elements into separate linear fibrils (Model 2; figure 7c) with tight packing and disulphide bonds, then the Voigt estimate can be applied (equation (7.2)):

$$E_c = V_1E_1 + V_2E_2 + \text{etc.} \quad (7.2)$$

This time the composite modulus E_c is higher, at 1.4 GPa. Although this modulus is 2–3 times higher than the observed stiffnesses, it is not compromised by the presence of microvoids as distal threads commonly are (Bairati & Vitellaro-Zuccarello 1976). A Reuss estimate for the proximal portion yields an E_c of 8 MPa, which is less than half the observed E of 20–50 MPa for proximal threads. Non-inclusion of the very significant proportion of non-collagenous matrix (34%) in proximal threads must contribute to the underestimation.

Neither model specifically addresses the nonlinear peculiarities of mechanical behaviour. These include yield and stress softening in the distal portion as well as strain stiffening in the proximal portion. Model 2, however, does offer a more plausible explanation for yield and recovery in the distal portion; at yield stress, metal chelates in the stiff poly[-D] chains would fail and transfer the load to intact poly[-NG] chains. These would extend with little initial resistance and, upon relaxation, recover their initial length (see stress-strain curve for distal portion in figure 1b). Future studies will examine this possibility in detail. Recent identification of von Willebrand A domains in PTMP and the shear-induced binding of these to type I collagen (Smith *et al.* 2000) suggest that this protein may contribute to the reversible strain-induced stiffening observed in proximal threads (figure 5b).

8. CONCLUSION

Byssus is essentially an array of elastomerically graded fibres that mussels produce to attach themselves to hard surfaces in their habitat and on which they depend to moderate the forces buffeting them. Gradients are most evident in the span of a byssal thread between the attachment plaque and the stem. Here, stiffness drops by a factor of ten. Correlated to this mechanical ramp-down is the graded distribution of two chimaeric collagens: preCol-D, and -P and an ungraded third, -NG. All share similar collagen and terminal domains. The flanking domains wedged between these, however, exhibit a distinct resemblance to three well-known structural paradigms: the crystalline β -sheets (D) and amorphous domains (NG) of spider silk as well as the β -spirals of elastic pentapeptides (P). The axial and lateral self-assembly of preCols during byssal thread formation results in a distribution in which preCols with β -sheets predominate distally, while those with β -spirals prevail proximally.

Living organisms produce a great variety of graded structures. Structures with graded porosities, for example, are particularly common in nature (Currey 1984; Amada 1995; Gibson & Ashby 1997). Graded structures, and the pathways for producing them, probably persist because of the adaptive advantages they offer. Given the complexity of biological structures, however, information about structure–function relationships remains difficult to assess at a molecular level. Byssal threads may represent a singular opportunity for gaining insights into the molecular nature of structural gradients. Such insights are also emerging from industry: FGMs are being increasingly utilized for their superior performance in the next generation of electronic, energy conversion and aeronautical materials (Kaysser & Ilschner 1995; Koizumi & Niino 1995). In these technologies, it is generally appreciated that gradient structures smooth stress distribution, reduce stress concentrations, eliminate stress singularities and, in most cases, improve bonding strength and fracture toughness (Erdogan 1995). Many of these advantages are manifestly suggested in the structure and performance of mussel byssus.

The authors thank E. Carrington, B. Gaylord and S. Rapoport for their comments and stimulating discussion.

REFERENCES

- Amada, S. 1995 Hierarchical functionally gradient structures of bamboo, barley, and corn. *Mater. Res. Soc. Bull.* **20**, 35–36.
- Bairati, A. 1991 The byssus of the mussel *Mytilus* from the molecules to the organ: functional performance resides in the ultrastructural assembly. In *Form and function in zoology* (ed. G. Lanzavecchia & R. Valvassori), pp. 163–177. Modena: Mucchi.
- Bairati, A. & Vitellaro-Zuccarello, L. 1974 The ultrastructure of the byssal apparatus of *Mytilus galloprovincialis*. Analysis of byssus by polarized light microscopy. *J. Submicrosc. Cytol.* **6**, 367–379.
- Bairati, A. & Vitellaro-Zuccarello, L. 1976 The ultrastructure of the byssal apparatus of *Mytilus galloprovincialis*. IV. Observations by transmission electron microscopy. *Cell Tiss. Res.* **166**, 219–234.
- Bell, E. C. 1998 Mechanical design of mussel byssus.

- Presented at the Annual Meeting of the Society of Experimental Biology, Edinburgh, UK.
- Bell, E. C. & Gosline, J. M. 1996 Mechanical design of mussel byssus: material yield enhances attachment strength. *J. Exp. Biol.* **199**, 1005–1017.
- Bella, J. & Berman, H. M. 2000 Integrin–collagen complex: a metal–glutamate handshake. *Structure* **8**, R121–126.
- Benedict, C. V. & Waite, J. H. 1986 Composition and ultrastructure of the byssus of *Mytilus edulis*. *J. Morphol.* **189**, 261–270.
- Brown-Augsburger, P., Tisdale, C., Broekelmann, T., Sloan, C. & Mecham, R. P. 1995 Identification of an elastin cross-linking domain that joins three peptide chains. *J. Biol. Chem.* **270**, 17 778–17 783.
- Burzio, L. A. & Waite, J. H. 2000 Cross-linking in adhesive quinoproteins: studies with model decapeptides. *Biochemistry* **39**, 11 147–11 153.
- Coombs, T. L. & Keller, P. J. 1981 *Mytilus* byssal threads as an environmental marker for metals. *Aquat. Toxicol.* **1**, 291–300.
- Coyne, K. J. & Waite, J. H. 2000 In search of molecular dovetails in mussel byssus: from the threads to the stem. *J. Exp. Biol.* **203**, 1425–1431.
- Coyne, K. J., Qin, X. X. & Waite, J. H. 1997 Extensible collagen in mussel byssus: a natural block copolymer. *Science* **277**, 1830–1832.
- Currey, J. 1984 *The mechanical adaptations of bones*. Princeton University Press.
- Erdogan, F. 1995 Fracture mechanics of functionally graded materials. *Mater. Res. Soc. Bull.* **20**, 43–44.
- Exposito, J. Y., D'Alessio, M., Solursh, M. & Ramirez, R. 1992 Sea urchin collagen evolutionarily homologous to vertebrate pro- $\alpha 2$ (I) collagen. *J. Biol. Chem.* **267**, 15 559–15 564.
- Gathercole, L. J. & Keller, A. 1975 Light microscopic waveforms in collagenous tissues and their structural implications. In *Structure of fibrous biopolymers* (ed. E. D. T. Atkins & A. Keller), pp. 153–175. London: Butterworths.
- Gibson, L. J. & Ashby, M. F. 1997 *Cellular solids*, 2nd edn. Cambridge University Press.
- Gosline, J. M., Guerette, P. A., Ortlepp, C. S. & Savage, K. N. 1999 The mechanical design of spider silks: from fibroin sequence to mechanical function. *J. Exp. Biol.* **202**, 3292–3305.
- Guerette, P. A., Ginzinger, D. G., Weber, B. H. F. & Gosline, J. M. 1996 Silk properties determined by gland-specific expression of a spider fibroin gene family. *Science* **272**, 112–115.
- Harris, B. 1980 The mechanical behaviour of composite materials. *Symp. Soc. Exp. Biol.* **34**, 37–74.
- Hayashi, C. Y. & Lewis, R. V. 1998 Evidence from flagelliform cDNA for the structural basis of elasticity and modular nature of spider silks. *J. Mol. Biol.* **275**, 773–784.
- Jordan, T. E. & Valiela, I. 1982 A nitrogen budget for the ribbed mussel and its significance in nitrogen flow in a New England salt marsh. *Limnol. Oceanogr.* **27**, 75–90.
- Kaysser, W. A. & Ilschner, B. 1995 FGM research activities in Europe. *Mater. Res. Soc. Bull.* **20**, 22–25.
- King, G., Brown, E. M. & Chen, J. M. 1996 Computer model of bovine type I collagen microfibril. *Protein Engng* **9**, 43–49.
- Kinloch, A. J. 1987 *Adhesion and adhesives: science and technology*. London: Chapman & Hall.
- Koide, M., Lee, D. S. & Goldberg, E. D. 1982 Metal and transuranic records in mussel shell, byssal threads and tissues. *Estuar. Coast. Shelf Sci.* **15**, 679–695.
- Koizumi, M. & Niino, M. 1995 Overview of FGM research in Japan. *Mater. Res. Soc. Bull.* **20**, 19–21.
- Knupp, C., Luther, P. K., Morris, E. P., Knight, D. P. & Squire, J. M. 1999 Partially systematic molecular packing in the hexagonal columnar phase of dogfish egg case collagen. *J. Struct. Biol.* **126**, 121–130.
- Lewis, R. V. 1992 Spider silk: the unraveling of a mystery. *Acc. Chem. Res.* **25**, 392–398.
- Lilius, G., Persson, M., Bülow, L. & Mosbach, K. 1991 Metal affinity precipitation of proteins carrying genetically attached polyhistidine tails. *Eur. J. Biochem.* **198**, 499–504.
- Lugt, H. J. 1983 *Vortex flow in nature and technology*. New York: Wiley.
- McDowell, L. M., Burzio, L. A., Waite, J. H. & Schaefer, J. 1999 REDOR detection of cross-links formed in mussel byssus under high flow stress. *J. Biol. Chem.* **274**, 20 293–20 295.
- Mascolo, J. M. & Waite, J. H. 1986 Protein gradients in byssal threads of some marine bivalve molluscs. *J. Exp. Zool.* **240**, 1–7.
- Mercer, E. H. 1952 Observations on the molecular structure of byssus fibres. *Austl. Mar. Freshwat. Res.* **3**, 199–204.
- Pareti, F. I., Niiya, K., McPherson, J. M. & Ruggeri, Z. M. 1987 Isolation and characterization of two domains of human von Willebrand factor that interact with fibrillar collagen types I and III. *J. Biol. Chem.* **262**, 13 835–13 841.
- Pikkarainen, J., Rantanen, J., Vastamäki, M., Lampiaho, K. & Kulonen, E. 1968 On collagens of invertebrates with special reference to *Mytilus edulis*. *Eur. J. Biochem.* **4**, 555–560.
- Price, H. A. 1981 Byssus thread strength in the mussel, *Mytilus edulis*. *J. Zool. Lond.* **194**, 245–255.
- Prockop, D. J. & Fertala, A. 1998 The collagen fibril: the almost crystalline structure. *J. Struct. Biol.* **122**, 111–118.
- Prockop, D. J., Kivirikko, K. I., Tuderman, L. & Guzman, N. 1979 The biosynthesis of collagen and its disorders. *New Engl. J. Med.* **301**, 13–23.
- Qin, X. X. & Waite, J. H. 1995 Exotic collagen gradients in the byssus of the mussel *Mytilus edulis*. *J. Exp. Biol.* **198**, 633–644.
- Qin, X. X. & Waite, J. H. 1998 A collagenous precursor that may mediate block copolymer gradients in mussel byssal threads. *Proc. Natl Acad. Sci. USA* **95**, 10 517–10 522.
- Qin, X. X., Coyne, K. J. & Waite, J. H. 1997 Tough tendons: mussel byssus has collagen with silk-like domains. *J. Biol. Chem.* **272**, 32 623–32 627.
- Rabin, B. H., Williamson, R. L. & Suresh, S. 1995 Fundamentals of residual stresses in joints between dissimilar materials. *Mater. Res. Soc. Bull.* **20**, 37–39.
- Rudall, K. M. 1955 The distribution of collagen and chitin. *Symp. Soc. Exp. Biol.* **9**, 49–71.
- Sachetto-Martins, G., Franco, L. & Oliveira, D. E. 2000 Plant glycine rich proteins: a family or just proteins with a common motif? *Biochim. Biophys. Acta* **1492**, 1–14.
- Sezutsu, H. & Yukuhiro, K. 2000 Dynamic rearrangement within the *Antheraea pernyi* gene is associated with four types of repetitive units. *J. Mol. Evol.* **51**, 329–338.
- Simmons, A. H., Michal, C. A. & Jelinski, L. W. 1996 Molecular orientation and two-component nature of the crystalline fraction of spider dragline silk. *Science* **271**, 84–87.
- Smeathers, J. E. & Vincent, J. F. V. 1979 Mechanical properties of mussel byssus threads. *J. Mollusc Stud.* **49**, 219–230.
- Smith, C., Estavillo, D., Emsley, J., Bankston, L. A., Liddington, R. C. & Cruz, M. A. 2000 Mapping the collagen-binding site in the domain of the glycoprotein Ia/IIa (integrin $\alpha 2\beta 1$). *J. Biol. Chem.* **275**, 4205–4209.
- Smith, M. C., Furman, T. C. & Pidgeon, C. 1987 Immobilized iminodiacetic acid metal peptide complexes. Identification of chelating peptide purification handles for recombinant proteins. *Inorg. Chem.* **26**, 1965–1969.
- Tamarin, A. 1975 An ultrastructural study of byssus stem formation in *Mytilus californianus*. *J. Morphol.* **145**, 151–177.
- Tamarin, A., Lewis, P. & Askey, J. 1976 The structure and formation of the byssus adhesive plaque in *Mytilus*. *J. Morphol.* **149**, 199–221.
- Taylor, S. W., Chase, D. B., Emptage, M. H., Nelson, M. J. &

- Waite, J. H. 1996 Ferric ion complexes of a DOPA-containing adhesive protein from *Mytilus edulis*. *Inorg. Chem.* **35**, 7572–7577.
- Vitellaro-Zuccarello, L., De Biasi, S. & Bairati, A. 1983 Ultrastructure of the byssal apparatus of a mussel. V. Localization of the collagenic and elastic components in the threads. *Tiss. Cell* **15**, 547–554.
- Vaccaro, E. & Waite, J. H. 2001 Yield and post-yield behavior of mussel thread: a self-healing biomolecular material. *Biomacromolecules* **2**, 906–911.
- Van Ness, K. P., Koob, T. J. & Eyre, D. R. 1988 Collagen cross-linking: distribution of hydroxypyridinium cross-links among invertebrate phyla and tissues. *Comp. Biochem. Physiol. B* **91**, 531–534.
- Waite, J. H. 1992 The formation of mussel byssus: anatomy of a natural manufacturing process. *Results Problems Cell Different.* **19**, 27–54.
- Waite, J. H., Saleuddin, A. S. M. & Andersen, S. O. 1979 Periostracin—a soluble precursor of sclerotized periostracum in *Mytilus edulis* L. *J. Comp. Physiol.* **130**, 301–307.
- Waite, J. H., Qin, X. X. & Coyne, K. J. 1998 The peculiar collagens of mussel byssus. *Matrix Biol.* **17**, 93–106.
- Wainwright, S. A., Biggs, W. D., Currey, J. D. & Gosline, J. M. 1976 *Mechanical design in organisms*. Princeton University Press.
- Wang, K., McCarter, R., Wright, J., Beverly, J. & Ramirez-Mitchell, R. 1993 Viscoelasticity of the sarcomere matrix of skeletal muscles. *Biophys. J.* **64**, 1161–1177.