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Mussel collagen molecules with silk-like domains as load-bearing elements in distal byssal threads

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ABSTRACT

Mechanically stressed biological materials like tendon, spider silk or mussel byssal threads are typically composite materials comprising multi-domain proteins, in which molecular building blocks contribute to overall material function.

Mussel byssal threads are the anchorage of sessile mytilid mussels, which withstand recurring external loads from waves and tides. A single thread is elastic and ductile proximally, while the distal portion exhibits an extraordinary stiffness and toughness with a transient gradient of both mechanical features along the thread. The main components of byssal threads include a set of various collagen-like structural proteins (preCols) consisting of a collagenous core sequence flanked by globular domains. Here, structural analysis using polarized Fourier-transform infrared spectroscopy (FTIR) on stretched distal portions of mussel byssal threads determines the impact of external linear load on various molecular moieties. It is concluded that the preCol collagenous core domain is the main load-bearing element in distal byssal threads, while polyalanine beta-sheets in the flanking domains, similar to those found in spider silk proteins, provide high stiffness at low strains. Load dissipation is mediated by domain stretching of amorphous glycine-rich helical moieties followed by complete unfolding of the preCol flanking domains.

1. Introduction

Nature has generated many high performance materials with astonishing properties. Among these outstanding materials is the mussel byssus, a unique anchorage system of some marine mussel species. Mussel byssi combine elastic and shock absorbing properties with high breaking strength and the capacity for self-healing. With a bundle of threads, sessile mussels hold fast to any kind of surface in their habitat and bear large loads both in dry and wet states when exposed to tidal changes and waves.

The mechanical properties and composition of byssal threads from the genus *Mytilus* have been extensively studied (Aldred et al., 2007; Bell and Gosline, 1996; Brazee and Carrington, 2006; Lucas et al., 2002; Smeathers and Vincent, 1979; Waite et al., 1998). Individual threads can be differentiated into proximal and distal portions, with the proximal part of the threads being elastic and the distal portion being more rigid and stiff. Notably, the distal portion exhibits unique mechanical properties with yielding, that enables efficient load dissipation prior to strain hardening (Bell and Gosline, 1996). The self-healing abilities of distal byssal threads are characterized by a time-dependent post-yield recovery after stress removal, increasing the life-time of each individual thread (Harrington and Waite, 2007; Vaccaro and Waite, 2001).

The mechanical properties of byssal threads originate in their composite structure, where collagenous fibrils are embedded into a proteinaceous matrix surrounded by a granular cuticle (Fig. 1). Solid state NMR and X-ray fiber diffraction studies on distal portions of byssal threads revealed tightly packed proteins with distinct triple helical and beta-structure elements oriented along the thread axis (Hagenau et al., 2009; Harrington et al., 2009; Mercer, 1952; Rudall, 1955). Sequence analysis of cDNAs derived from the mussel foot (Lucas et al., 2002; Waite et al., 1998), the organ in which byssal proteins are produced, showed that the main proteins of the byssal thread are the so-called preCols (Coyne et al., 1997; Qin and Waite, 1995, 1998; Qin et al., 1997). The preCols consist of a collagenous core domain with the highly repetitive amino acid sequence motif Gly-Xaa-Yaa (Xaa usually denotes proline or glycine, Yaa hydroxyproline) typical for collagens in combination with specific non-collagenous carboxyl- and amino-terminal flanking regions containing amino acid motifs typically found in structural proteins such as elastin and spider dragline silk (Scheibel and Serpell, 2005). The flanks are thought to mediate the mechanical properties of the respective proximal and distal portions of the thread (Harrington and Waite, 2007; Waite et al., 1998).

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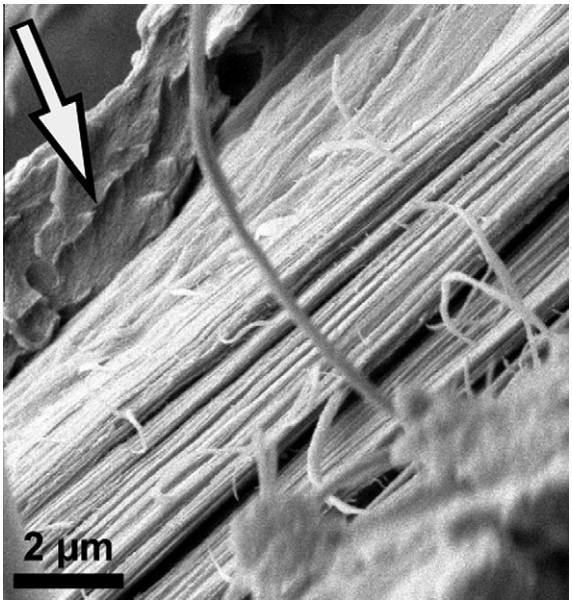

Fig. 1. SEM image of a distal mussel byssal thread of *Mytilus galloprovincialis* (magnification 16.000×). The distal thread core consists of fibrils aligned straight along the thread axis covered by a granular cuticle (arrow).

The flank sequences of preCol P, the protein mainly found in the proximal portion of the thread, resemble features of elastic fibrous proteins like elastin and spider flagelliform silk (Coyne et al., 1997; Fu et al., 2009; Scheibel, 2004), whereas the flank sequences of the main component of the stiff distal thread portion, preCol D, contain repeated polyalanine runs also found in spider dragline silk, an extremely tough material. In dragline silk, these repeats form blocks of beta-pleated sheets being important for mediating the materials stiffness (Hardy et al., 2008; Keten et al., 2010; Papadopoulos et al., 2009b; van Beek et al., 2002). Additionally, preCol D contains clusters of $(Gly\text{-}Gly\text{-}Xaa)_n$ (Xaa denotes mainly leucine, but also alanine and valine) and Gly-Xaa-Gly-Xaa motifs which adopt helical (3_1 helix, polyglycine II structure) and beta-sheet conformations (polyglycine I structure) in silkworm and spider dragline silk (Fu et al., 2009; Scheibel, 2004). A third preCol is found homogenously distributed along the entire thread and is therefore called preCol NG (for non-gradual). Here, the flanking regions contain polyalanine blocks, $(Gly\text{-}Gly\text{-}Xaa)_n$ clusters and Gly-Xaa-Gly-Xaa motifs similar to preCol D, but with a higher content of polyglycine blocks forming helical conformations. These sequences resemble spider dragline silk proteins as well as glycine-rich proteins from plant cell-walls (Condit and Meagher, 1986; Qin and Waite, 1998).

Histidine and DOPA (di-hydroxyphenylalanine)-rich sequences terminate the flanking regions of all preCols. It is proposed that they provide sites for lateral and longitudinal cross-linking (Waite et al., 1998, 2002).

In spite of a large amount of information on the mechanical properties and the composition of byssal threads, no structural model is available which reveals the molecular effects of external load on structure until thread rupture. Recently, studies combining mechanical and time-resolved infrared spectroscopy on spider dragline and minor ampullate silk have allowed the analysis of the structural organization and the response of different structural moieties to external mechanical fields, revealing a serial connection of crystalline polyalanine blocks and pre-strained amorphous chains (Ene, 2009, 2010; Papadopoulos et al., 2007; Papadopoulos, 2009a,b). Here, we analyzed the influence of mechanical stress on the distal portion of the byssal thread using this methodology. In particular, the orientation of the single building blocks and their molecular response to applied external linear load were characterized as a fundamental basis to provide a structural model on load bearing and dissipation in distal byssal threads.

2. Materials and methods

2.1. Sample preparation

Marine mussels (*Mytilus galloprovincialis*, Lamarck) originated from a breeding farm in Goro (Veneto, Italy) were washed, and the byssi were removed from the mussel. The distal portions of individual byssal threads were prepared by cutting near the plaque and the proximal region. The distal threads were either incubated in deionized water (DW) or artificial sea water (ASW, 480 mM NaCl, 28 mM $MgSO_4 \times 7\ H_2O$, 24 mM $MgCl_2 \times 6\ H_2O$, 16 mM $CaCl_2 \times 6\ H_2O$, 2.4 mM $NaHCO_3$) at 4 °C. Incubation in deionized water was used to remove salt ions in the byssal threads. Before FTIR measurements, the threads incubated in ASW were rinsed in deionized water to remove residual salt on the surface of the thread. To gain dehydrated threads they were dried in air (T = 21 °C, RH = 21%). The elliptical cross sectional area of each thread was determined by measuring the major (a = 90–200 µm) and minor (b = 40–60 µm) axis of the thread with an optical microscope and calculated as follows:

$$A_0 = \pi ab/4 \tag{1}$$

2.2. Stress–strain measurements

The distal threads were adjusted to an initial sample length of 2 mm and then attached to plastic frames using water resistant glue (UHU plast Kunststoff Modellbau, UHU). The force measurements were performed with a Bose ElectroForce 3220 (Bose ElectroForce Systems Group, Bose Corporation) equipped with a 250 g load cell and a humidity chamber suitable for under water studies. The strain rate was 0.1 mm sec^{-1}. Dehydrated threads were measured in air (T = 21–23 °C, RH = 25–30%) and hydrated threads in DW or ASW. In spite of a high sample number, only 7–9 measurements for each experimental set were considered reliable for further analysis.

Stress and strain were determined from applied load and thread length as follows:

$$\varepsilon = \Delta L/L_0 \tag{2}$$

$$\sigma = F/A_0, \tag{3}$$

where ε is the nominal strain, σ the engineering stress, F the tensile load applied to the thread, L the thread length, L_0 the initial thread length and A_o the initial cross sectional area. Due to thread thinning at a constant volume during elongation, true stress–strain curves were calculated:

$$\varepsilon_t = 1n(1+\varepsilon) \tag{4}$$

$$\sigma = \sigma_t(1+\varepsilon), \tag{5}$$

where ε_t and σ_t are the true strain and true stress, respectively.

E-modulus (Youngs modulus) was obtained by determining the slope of the linear-elastic part of the stress–strain curve. The yield offsets were determined by the intersection of curve tangents in the respective region, and toughness was obtained by integrating the entire curve.

2.3. Combined micromechanics and FTIR measurements

FTIR studies of threads under tension were performed using a measurement setup (Fig. 5a) as described in previous studies