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The modulus of elasticity of lobster aorta microfibrils

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Abstract. The presence of elastic fibres in the extracellular matrix (ECM) provides physiologically important elastic properties for many tissues. Until recently, microfibrils, one component of the ECM, were thought primarily to serve as a scaffolding on which elastin is deposited during development to form elaunin fibres [1]. The most prominent protein that forms mammalian microfibrils is fibrillin. It is known that mutations in the fibrillin gene cause a heterogenous connective tissue disease called Marfan syndrome [2], so information on mechanical properties of microfibrils or their role in tissue function would be useful. Microfibrils are also found in the ECM of some invertebrate tissues, and there is growing evidence that the protein forming the structure is homologous to mammalian fibrillin [3, 4]. It has been shown that the microfibril-based arterial wall of the lobster has viscoelastic properties [5], and we have now utilized this primitive artery to measure the modulus of elasticity of microfibrils. It is similar to that of the rubber-like protein elastin.
Key words. Artery; aorta; elastin; extracellular matrix; lobster; Marfan syndrome; microfibrils.

In most circulatory systems, arteries located close to the heart provide functionally important elastic behaviour. In vertebrate arteries this elastic behaviour has been attributed to the distinct properties of the two principal fibres composing the structures. At low strains, the compliant elastin fibres are deformed, but as the strain increases, collagen fibres, which are kinked at low strains, are straightened. At high strains, the stiff collagen fibres are directly stressed and increase the modulus of the whole wall. Because of the sequential deformation of first the compliant elastin, and then the stiff collagen fibres, the arterial wall shows nonlinear viscoelastic behaviour.

The arterial wall of some invertebrates also shows nonlinear viscoelastic behaviour, although the composition of the wall is based mostly on microfibrils that have a strong resemblance to those found in mammalian tissues [5]. The microfibrils found in the arterial wall of the lobster *Homarus americanus* are about 20–35 nm in diameter, compared with mammalian microfibrils, which typically have a diameter of about 10–12 nm. The lobster arterial microfibrils have a beaded appearance that provides a distinct periodicity (fig. 1), which is a condition that is also found in mammalian microfibrils. The periodicity of the unstresssed lobster arterial microfibrils is about 43 nm and has been observed to increase to approximately 58 nm under stress. These measurements are less than those for mammalian microfibrils, which when unstressed have a periodicity of about 52 nm and stressed have a periodicity of about 75 nm [6].

The cylindrical geometry of an artery, and the simple architecture of the wall of the lobster abdominal artery, provide a unique opportunity to make measurements of the mechanical properties of individual microfibrils. We have shown (in preparation) that the nonlinear elastic behaviour of lobster arterial wall can be completely described by accounting for the reorientation (at low strains) and then deformation (at high strains) of the microfibrils. This is in itself of interest, since it provides a new evolutionary solution to the functional requirement that pressurized elastic-walled vessels have nonlinear elasticity. These calculations required knowledge of the mechanical properties of individual microfibrils that we present here.

Materials and methods

Specimens of *H. americanus* were purchased locally, and kept in 200-l tanks of recirculated seawater at 10 °C. Animals were sacrificed by bubbling CO_2 into a container of seawater for 20 min. The carapace was removed posterior to the cervical groove, and the abdominal segments were cut laterally and removed. The abdominal extensor muscles were separated and reflected to expose the abdominal aorta. The section between the first and second lateral arteries was excised with the junction of the first lateral artery intact to orient the section. A 2- to 5-mm ring was cut from the section's distal end, and the ring's wall was cut, provid-

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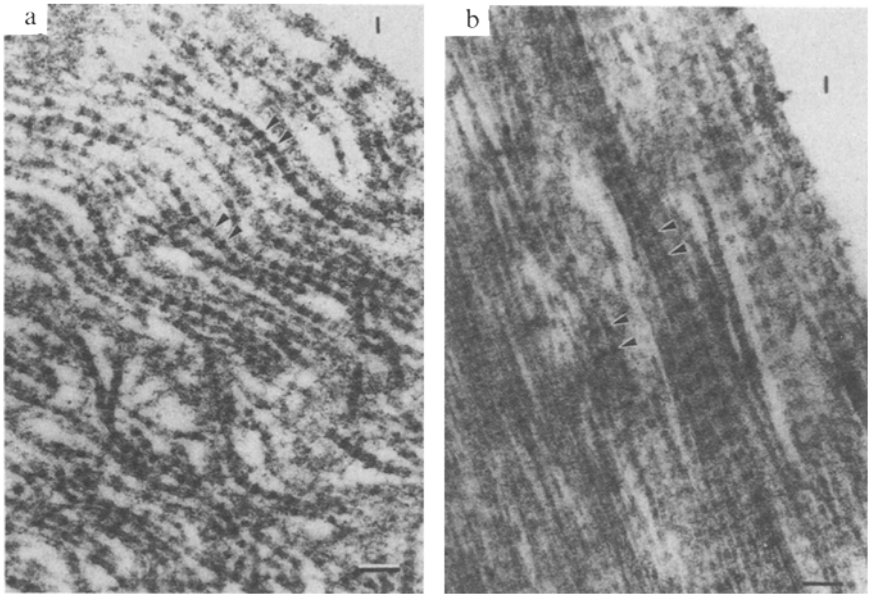

Figure 1. Electron micrographs of the dorsal abdominal artery of the lobster for samples (*a*) at zero stress and (*b*) stressed with an intraluminal pressure of 3.75 kPa. Note the beaded periodicity (arrowheads) and differences in orientation of microfibrils relative to the lumen (l). Scale bars: 100 nm. The volume fraction of microfibrils in the wall is 0.317 ± 0.07 (SE). (Note: the volume fraction measurement includes the entire vessel wall, not just the microfibril bundles shown.)

ing a zero-stress sample [7]. The vessel was tied with silk thread between two blunted 18-gauge syringe tips, and submerged in a perfusion bath. One syringe tip was plugged, and the other was connected to a reservoir. A pump circulated perfusate from the bath to the reservoir to maintain a constant pressure head. Overflow from the reservoir returned to the bath via a downspout. Transmural pressure was controlled by varying the height of the pressure head. The syringe tips held the section at approximately 90% of its in situ length. This section length was about 15 times the cannula diameter, and all electron microscopy sections were taken from the centre of the vessel section. The zero-stress sample floated in the reservoir. Sections were prefixed for 20 min in 0.1% OsO_4 before fixation for 1 h with 2.5% glutaraldehyde, both in seawater at 10 °C, for transmural pressures of 1.30, 2.03, 2.64, 3.56 and 3.75 kPa.

Electron micrographs (EMs) were taken using a Hitachi H600 electron microscope operating at 75 kV at a magnification of 95,000×. The micrographs were analyzed with Bioquant for OS/2 (R&M Biometrics Inc., 561 Ohio Ave, Nashville, TN 37209). This software was used to digitize the micrographs and to make all measurements.

A microfibril's length was defined as the distance from the leading edge of one 'bead' to the leading edge of the next on the string. The chord lengths of sequential microfibrils were measured such that

$$nL \approx 100$$

where $n = 2$, 3, 4, 5 and L is the number of replicates: i.e. the leading edge of one bead to the leading edge of the second down the string ($2L$) was measured 50 times per micrograph, the leading edge of one bead to the leading edge of the third down the string ($3L$) was measured 33 times, and so on.

The length measurements were plotted against the number of sequential microfibrils (n), and a linear regression was performed with the intercept forced to the origin to find the length of an individual microfibril (fig. 2). The regression analysis accounted for the replication in the y-axis values [8]. Measurements from all zero-stress samples were pooled before performing the regression to find the resting length. The strain on an individual microfibril is then calculated as

$$\varepsilon = \ln\left(\frac{L_p}{L_o}\right)$$

where L_p is the length of the microfibril at some known transmural pressure, and L_o is its resting length.

The ratio of the vessels' midwall radii to their thicknesses averaged 19.5 ± 5.1, so the thin-walled pressure vessel approximation was used for stress calculations. Thus, circumferential wall stress was determined using

$$\sigma_c = \frac{Pr_{mw}}{t} \qquad (1)$$

where σ_c is circumferential stress in the wall, P is transmural pressure, r_{mw} is the vessel's midwall radius and t its thickness. The tensile stress on an individual microfibril will not, however, be the circumferential stress acting on the vessel wall. Since the wall is not made purely of microfibrils, the stress acting on each component is proportional to the fraction of the whole it occupies. In general,

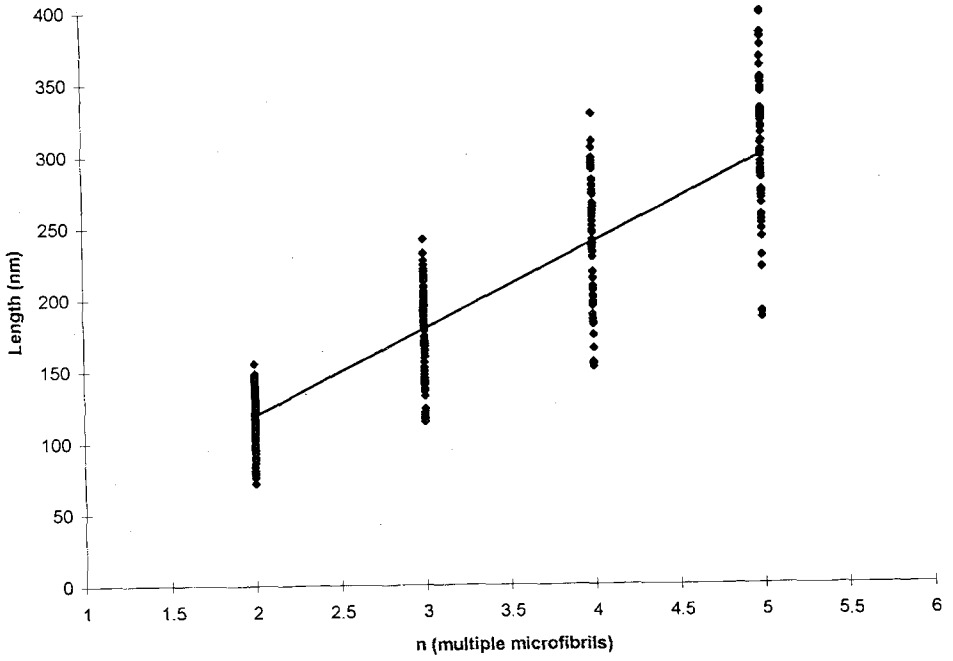

Figure 2. An example of the data used to measure the length of a stressed microfibril. See text for an explanation of data analysis. These measurements were made from an EM of the wall of a vessel pressurized to 3.75 kPa. Micrographs were digitized, and all measurements were made using Bioquant for OS/2. The slope of the regression line is 59.94 ± 3.10 (SE) nm ($r^2 = 0.974$).

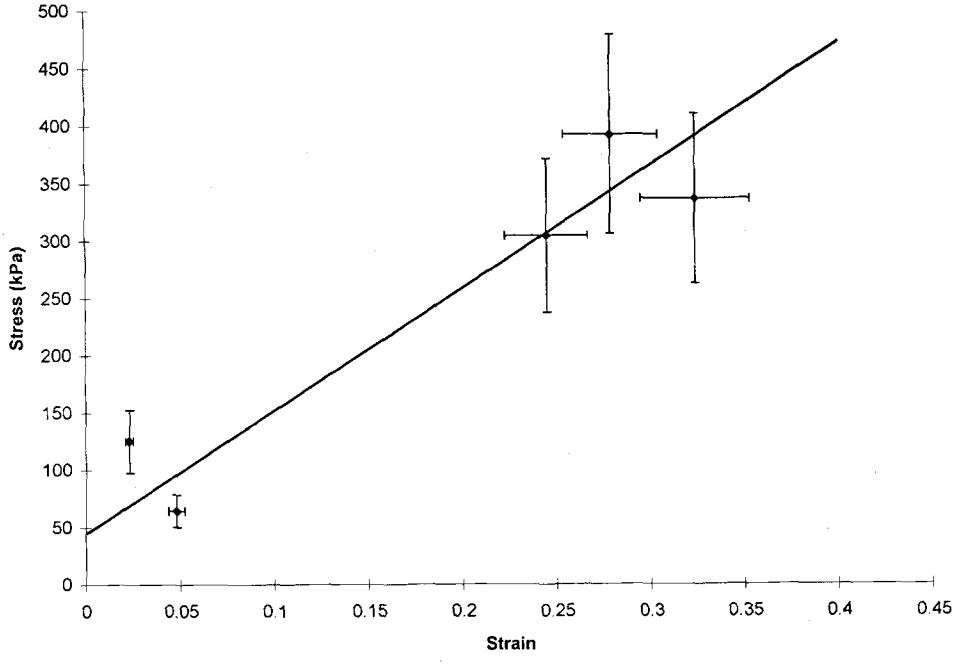

Figure 3. A graph of stress vs strain for lobster artery microfibrils. Error bars for stress and strain denote an estimate of experimental uncertainty and propagated standard errors of the regression slopes (from fig. 2), respectively. The slope [11, 12] of the regression line is 1.06×10^6 Pa, and represents the modulus of elasticity of the microfibrils.

$$\sigma_t = V_f\sigma_f + (1 - V_f)\sigma_m \quad (2)$$

where σ_t is the stress on a tissue, V_f is the volume fraction of the tissue occupied by reinforcing fibres, σ_f is the stress on the fibres and σ_m is the stress on the matrix in which they are embedded. It is explicitly assumed that the matrix bears no load in tension, so the stress acting on the fibres is

$$\sigma_f = \frac{\sigma_t}{V_f} \quad (3)$$

Setting σ_t equal to σ_c, and substituting in equation 1 gives

$$\sigma_f = \frac{Pr_{mw}}{V_f t} \quad (4)$$

which is the quantity shown on the y-axis in figure 3. Microfibrils are not distributed uniformly but are aggregated into bundles which are approximately uniform across the vessel wall. To measure the volume fraction in a bundle, a line was marked on the digitized electron micrograph, and the ratio of its length covered by microfibrils determined, four times per micrograph. This procedure was carried out on toluidine blue-stained thick sections, cut from the same resin block as the EM thin sections, to measure the volume fraction of bundles through the wall, 10 times per thick section. The product of these two ratios approximates the volume fraction of microfibrils in the wall.

Results and discussion

We do not know the molecular components of the microfibrils found in lobster arterial wall; however, the ECM microfibrils in tissues of two more primitive invertebrates, the mesoglea of the jellyfish *Podocoryne carnea* [4], and the dermis of the sea cucumber *Cucumaria frondosa* [3], are homologous to mammalian fibrillins. Mechanical studies on the mesoglea of another jellyfish, *Polyorchis penicillatus*, has already shown that the modulus of elasticity of the elastic radial fibers, which are presumably the same as the microfibrils described for *P. carnea*, is about 1 MPa and provides important elastic properties to the whole tissue [9]. This estimate was based on morphological data and mechanical measurements of fresh whole mesoglea. The modulus of elasticity of microfibrils found in the abdominal artery of the lobster *H. americanus* is 1.06 ± 0.217 MPa. This compares quite favourably with the modulus of elastin, which is about 1.2 MPa [10].

This work shows that microfibrils display linear deformation behaviour, and since there is growing evidence that there is structural conservation in fibrillin from cnidarians to humans, this value probably repre-sents the modulus of elasticity of other microfibrils. The knowledge that microfibrils provide mechanical behaviour will be important in assisting research to determine the mechanism and cure for connective tissue diseases such as Marfan syndrome.

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1 Montes G. S. (1992) Distribution of oxytalan, elaunin and elastic fibres in tissues. J. Brazilian Assoc. Adv. Sci. **44:** 224–233
2 Ramirez F. L., Pereira L., Zhang H. and Lee B. (1993) The fibrillin-Marfan syndrome connection. BioEssays **15:** 589–594
3 Thurmond F. A., Koob T. J. and Trotter, J. A. (1995) The microfibrils of sea cucumber dermis are similar to fibrilin microfibrils and form a network that has long range elasticity. Molec. Biol. Cell **6** (Supp.): 380a
4 Ruber-Muller S., Spissinger T., Schuchert P., Spring J. and Schmid V. (1995) An extracellular matrix protein of jellyfish homologous to mammalian fibrillins forms different fibrils depending on the life stage of the animal. Devl Biol. **169:** 662–672
5 Davison I. G., Wright G. M. and DeMont M. E. (1995) The structure and physical properties of invertebrate and primitive vertebrate arteries. J. Expl Biol. **198:** 2185–2196
6 Keene D. R., Maddox B. K., Kuo H. J., Sakai L. Y. and Glanville R. W. (1991) Extraction of extendable beaded structures and their identification as fibrillin-containing extracellular matrix microfibrils. J. Histochem. Cytochem. **39:** 441–449
7 Liu S. Q. and Fung Y. C. (1988) Zero-stress states of arteries. J. Biomed. Eng. **110:** 82–84
8 Zar J. H. (1984) Biostatistical Analysis, 2nd ed., Prentice Hall, Englewood Cliffs, NJ
9 DeMont M. E. and Gosline J. M. (1988) Mechanics of jet propulsion in the hydromedusan jellyfish, *Polyorchis penicillatus*. I. Mechanical properties of the locomotor structure. J. Exp. Biol. **134:** 313–332
10 Aaron B. B. and Gosline J. M. (1981) Elastin as a random-network elastomer: a mechanical and optical analysis of single elastin fibers. Biopolymers **20:** 1247–1260
11 Reed B. C. (1989) Linear least-squares fits with errors in both coordinates. Am. J. Physics **57:** 642–646
12 Reed B. C. (1992) Linear least-squares fits with errors in both coordinates. II. Comments on parameter variances. Am. J. Physics **60:** 59–62