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DYNAMIC MECHANICAL PROPERTIES OF EXTENSOR MUSCLE CELLS OF THE SHRIMP *PANDALUS DANAE*: CELL DESIGN FOR ESCAPE LOCOMOTION

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Summary

We measured the dynamic stiffness of passive single extensor cells from the abdomen of the shrimp *Pandalus danae*. These stiffness measurements, consisting of both elastic and viscous moduli, were carried out by imposing sinusoidal length changes on single cells and recording, simultaneously, the resultant force. Our measurements were made over a broad range of loading frequencies (1–120 Hz) and sarcomere lengths (1.9–3.2 μm), encompassing the physiological range of these parameters.

We found that: (1) there is an exponential increase in both elastic and viscous moduli with increases in sarcomere length; (2) there is a weak increase in these moduli with increasing frequency; (3) the relative energy dissipated by the viscous behavior of cells is largest in the relevant physiological range of loading frequencies and sarcomere lengths, with the magnitude of the viscous modulus nearly equal to the elastic modulus; and (4) the energy dissipated by the viscous behavior of these cells alone constitutes about 10 % of the total energy required to accelerate the animal in its aquatic environment. We propose that such large values for viscous energy dissipation in passive cells may be necessary to prevent unstable responses of muscle to the rapid dynamic loads imposed by the escape event.

Introduction

Studies of aquatic locomotion have focused on understanding how the morphology of an organism and the kinematics of its propulsor affect some measures of performance such as thrust, speed, acceleration, efficiency or energy consumption (for reviews, see Weihs and Webb, 1983; Daniel and Webb, 1987). By prescribing the motion of a propulsor such as a fin or some other portion of a body, hydrodynamic theories are directed towards predicting thrust and energy requirements (Lighthill, 1975; Wu, 1975; Webb, 1984; Blake, 1981; Nachtigall, 1980). Such approaches, while providing novel hypotheses about how an animal's morphology or the kinematics of its propulsor may minimize energy requirements

Key words: muscle, mechanics, passive elasticity and viscosity, shrimp, *Pandalus danae*.

for movement (see, for example, Weihs, 1974, 1980; Wu, 1971), neglect any physical or physiological limitations on actual appendage movements. We can, however, envisage several potential limitations that have been unexplored for aquatic locomotion and only recently examined in other locomotor systems. For example, there may be limits imposed upon the maximum stress produced by muscles involved in generating propulsive forces (e.g. muscle stress is presumed to be constant for a particular muscle type). Indeed, Biewener *et al.* (1988) and Perry *et al.* (1988) showed that the stresses in normal locomotion may rarely reach the limits suggested by isolated cell or muscle preparations. In addition, Daniel and Meyhöfer (1989) showed that a presumed constant value for muscle stress in abdominal flexors of the shrimp sets an upper size limit to escape locomotion. Maximum isometric stress may therefore play a crucial role in limiting the ranges and rates of propulsor movements.

It is also possible that other mechanical factors in locomotor systems may constrain the magnitude of useful work done by propulsive muscles or the range of body movements that are physiologically feasible. For example, we know remarkably little about how the mechanical properties of muscle affect overall energy requirements for aquatic locomotion. We need to know (i) the capacity of muscle for storing elastic strain energy, (ii) the extent to which muscle may dissipate any energy during passive extension and active contraction and (iii) how these two parameters may vary over the physiological range of sarcomere lengths or rates of length change. Only with this information can we more fully understand the mechanics and energetics of aquatic locomotion.

For many cases we have evidence that the mechanical properties of muscle may reflect its functional role in locomotion. For example, numerous studies suggest that the capacity of tissues, especially tendon, to store elastic strain energy may reduce the energy cost for locomotion for a variety of terrestrial organisms (Alexander and Bennet-Clark, 1977; Alexander *et al.* 1982; Biewener *et al.* 1981; Cavagna *et al.* 1977). Although muscle as a site of energy storage has received less attention, Tidball and Daniel (1986) showed that there is a large capacity for energy storage in frog semitendinosus muscle, but their data also indicated that these muscle fibers dissipate, by viscosity, a large fraction of the energy imparted to them. This dissipation reached a distinct peak in the middle of the physiological range of sarcomere lengths. The implications of such high losses of mechanical energy remain unexplored.

While the mechanical properties of muscle have been a subject of intense research for several decades, the emphasis has been one of understanding the molecular mechanisms associated with force generation (for reviews, see Gordon, 1989; Sugi and Pollack, 1988). Mechanical measurements are therefore directed towards determining the presumed rates and mechanics of cross-bridge attachment, the time course of force generation, or the relationships between force and speed of contraction. Much of this work either neglects the passive properties of muscle or presumes that they contribute no time-history to the mechanical behavior of muscle. With the exception of some of the earliest work on visco-

elastic properties of skeletal cells (e.g. Buchthal and Rosenfalck, 1957), we know remarkably little about how the dynamic mechanical properties of muscle, even in the passive state, may affect energy requirements for locomotion. To define potential physical limits to locomotion we need to quantify both the capacity of muscle to store elastic strain energy and the amount of energy that may be viscously dissipated.

In this paper, we examine how the mechanical properties of individual muscle cells in the abdomen of the common shrimp, *Pandalus danae* Stimpson, may affect one extreme mode of locomotion: the rapid escape response. The muscles associated with this propulsive scheme constitute a significant fraction of the mass of the animal (about 40 %); about one-third of them are partially extended in the passive state. Our goals are to determine (i) how the mechanical properties of muscle cells vary over a broad range of loading frequencies and sarcomere lengths and (ii) how these properties may affect the fraction of the total available energy delivered to the escape process.

Materials and methods

Animals, motions and morphology

We used the shrimp *Pandalus danae* collected either from the docks and floats around the University of Washington Friday Harbor Laboratory during the summer or by otter trawl around Lopez Island, Washington, during the winter. Animals were kept in a flow-through seawater system at 12 °C.

The physiological range of loading frequencies was estimated from high-speed ciné films of escaping shrimp (Daniel and Meyhöfer, 1989). The duration of the tail-flip and the angle of the tail with respect to the abdomen were measured by sequential frame analysis. The period of loading was estimated either from the total duration of the tail-flip or from the first half of that movement, as the first and second halves of the tail-flip do not have identical times.

Cell isolation

Each individual was decapitated and its abdomen was isolated and placed in cooled crustacean Ringer's solution (see Table 1). The dorsal aspect of the carapace was removed, exposing the set of abdominal extensors. The two lateral halves of this muscle group, consisting of about 10 cells each, were then placed in an intracellular skinning solution containing 0.5 % Triton X-100 to solubilize the membrane, giving us control over internal calcium and ATP concentrations. With iridectomy scissors, a single extensor muscle cell was then mechanically dissected away from its neighboring cells and placed in a relaxing solution containing ATP (Table 1). Since there are so few cells in the muscle group, we were able to use the homologous cells for every individual. We used two cells: one was relatively small (C1, 0.2 mm^2 cross-sectional area and 0.5 cm long) and is oriented more laterally and the other was larger (C2, 1 mm^2 cross-sectional area and 0.5 cm long) and located more medially in the tail.

Table 1. *The three solutions used in the experiments*

	Concentrations ($mmol\,l^{-1}$)									
	Total Na^+	Total K^+	Free Mg^{2+}	Free Ca^{2+}	Total EGTA	Total ATP	Total Hepes	Sucrose	Triton X-100	pH
Ringer's solution	440	11	2	10			10			7.4
Skinning solution	20	140	2	10^{-7}	50		50	500	0.5%	7.2
Relaxing solution	20	140	2	10^{-7}	50	5.78	20	500		7.2

The three solutions were a crustacean Ringer in which initial dissection and isolation of abdominal extensors was performed, a skinning solution that solubilizes the membranes of cells to create a preparation in which we could control intracellular levels of Ca^{2+} and ATP, and a relaxing solution in which ATP and Ca^{2+} levels were set to a value that prevents cross-bridge formation.

The composition of these solutions was calculated using the approach outlined by Fabiato and Fabiato (1979). The absolute stability constants for the relevant complexes of ligands and metals were taken from their paper.

For all solutions, magnesium and calcium were added as chloride salts, sodium and potassium were added as chloride salts to the Ringer and hydroxides to the skinning and relaxing solutions. The di-potassium salt of ATP was used.

Mechanical testing apparatus

A clear plastic chamber containing the relaxing solution was placed on a copper plate cooled to 12–15 °C by a Peltier element. The plate formed part of the stage of a modified compound microscope (Zeiss) used for mechanical measurements (Fig. 1). Two micromanipulators, attached to the stage, held a force transducer and a device for creating length changes of the cell.

Single cells were mounted between two clamps each made of a pair of needles ground flat and pressing against one another. One needle pair was attached to a force platform and the other to a piezo-electric crystal. Depending on the cell type, we used one of two force platforms, each consisting of a piece of stainless steel 8 mm wide and 5 mm long to which a needle pair was attached. Semiconductor strain gauges (Entran), mounted on both sides of the beam, formed two arms of a Wheatstone bridge, excited at 2.0 V, whose output was amplified with standard op-amp circuitry. Both force transducers were calibrated by suspending known weights from the tip of the needle pair attached to that device. Their frequency response was always greater than 150 Hz. Test samples of small pieces of rubber or stainless-steel wire yielded no measurable phase lag at frequencies less than 150 Hz. At that frequency, however, significant ringing was seen in the transducer. The platform used for the smaller C1 cells was 0.1 mm thick with a sensitivity of $12.5\,mV\,\mu N^{-1}$ and a compliance of $0.02\,m\,N^{-1}$. For the larger C2 cells we used a platform 0.2 mm thick with a sensitivity of $2.27\,mV\,\mu N^{-1}$ and a compliance of

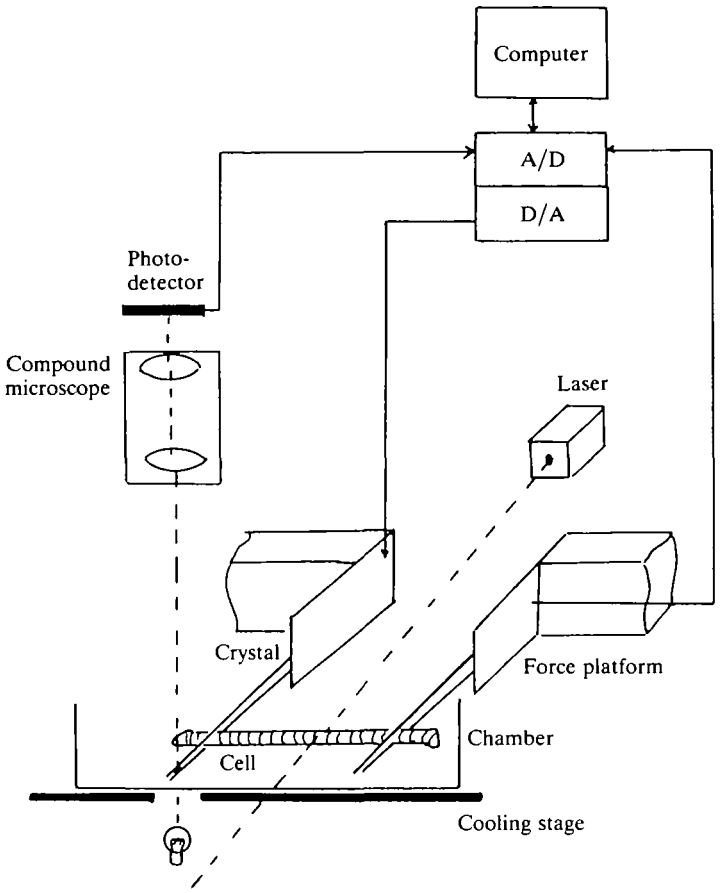

Fig. 1. A schematic diagram of the apparatus used in the experiments.

$0.0045\,m\,N^{-1}$. Sinusoidal length changes of the cell were driven by a computer-controlled voltage oscillator whose output deflected the piezo-electric crystal. With the output port of a data acquisition board (Data Translations) in the computer, we programmed a variety of loading frequencies from under 1 Hz to about 120 Hz. The instantaneous tensile force along the cell, encoded as a voltage from the bridge amplifier for the force transducer, was recorded on the computer with the analog port of the data acquisition board. We oscillated the cell for 20 cycles at each frequency and recorded 50 instantaneous force values during each cycle of oscillation. In general, both cell types were exposed to strains under 1%. The typical strain was approximately 0.6%.

The instantaneous length of the cell was monitored with two devices. First, we tracked the instantaneous position of the tip of the needle pair attached to the piezo-electric crystal with a diffuse silicon light detector (United Detector Technology). This detector, positioned at the end of a camera tube attached to the microscope, provided a current directly proportional to the position of the needle

image on its surface. The tip of the needle pair, imaged through a 40× objective and a 10× ocular, was projected onto the sensitive surface of the photodetector. This optical method, along with a transimpedance amplifier for the photodetector current, yielded displacement measurements with a sensitivity of 43.5 mV μm^{-1} and a frequency response of 10 kHz. The accuracy of the displacement measurement was limited by the bit resolution of the data acquisition board. This optical sensor was calibrated with an ocular micrometer.

The second component of length measurement came from the force transducer. Since this device moved in response to oscillatory tension on the cell, we had to account for its displacement in computing the overall deformation of the cell. We calibrated the displacement of the needle pair attached to the force transducer with an ocular micrometer, recording the voltage from the bridge amplifier. The instantaneous length of the cell was computed from the difference in the displacements of the needle pair attached to the piezo-electric crystal and the force transducer.

Sarcomere lengths and cell dimensions

The cell, clamped between the crystal and the force transducer and immersed in relaxing solution, was extended to a pre-selected sarcomere length with micromanipulators. Its overall length was measured with an ocular micrometer. The sarcomere length was measured with standard laser diffractometry (Zite-Ferenczy *et al.* 1986) using a He–Ne laser (Metrologic) passing through the center of the cell. The beam width of the laser was 0.5 mm, and it sampled approximately 10 % of the length of the cell.

At the end of each experiment, cells were fixed for electron and light microscopy (i) to verify the sarcomere length estimated from the above method, (ii) to determine the potential physiological range of sarcomere lengths from measured myofilament lengths and (iii) to determine the cross-sectional area of the cell. The latter measurement was accomplished by digitizing *camera lucida* tracings of the cross-section of each cell.

Our fixation procedure was as follows. After the mechanical tests of an individual fiber had been completed, the cells retained in the testing apparatus were fixed by immersion in 5 % glutaraldehyde buffered with 0.1 mol l^{-1} sodium cacodylate to pH 7.2 and containing 500 mmol l^{-1} sucrose. The cells were fixed for at least 20 min before they were removed from the clamps and stored for further processing in a fresh volume of fixative at 5 °C. Cells were washed in 0.1 mol l^{-1} sodium cacodylate buffer with 500 mmol l^{-1} sucrose and postfixed for 1 h in the same solution containing 1 % osmium tetroxide. The postfixative was washed out in several changes of 0.1 mol l^{-1} cacodylate buffer and distilled water and then rapidly dehydrated in a graded series of ethanol. Each fiber was cut into two parts and embedded in a standard mixture of Epon (Burke and Geiselmann, 1971). One part was used to obtain a cross-section for estimating cell areas by light microscopy and the other part was sectioned for transmission electron microscope (TEM) observations. Thin sections were cut with a diamond knife (Diatome) perpendicu-

lar to the long axis of the fiber to avoid compression. They were stained with uranyl acetate and lead citrate in the usual manner and viewed with a Philips 300 TEM.

Interpretation of mechanical data

The instantaneous voltage signals from the force transducer and the photodetector, along with their calibrations, were used to create a data set consisting of instantaneous cell length [$l(t)$] and tensile force [$F(t)$] for a variety of sarcomere lengths and a variety of frequencies of length change. These values were then converted to instantaneous stress and strain values using:

$$\sigma(t) = F(t)/A\,, \tag{1}$$

$$\epsilon(t) = l(t)/l_0\,, \tag{2}$$

where A is the cross-sectional area of the cell and l_0 is its initial length.

Since the stress and strain vary sinusoidally, their amplitudes are readily computed with a root-mean-square method. The phase between stress and strain was determined algebraically from the amplitudes of these two waves and the amplitude of the wave that is formed by their difference. The total dynamic stiffness (E^*) is given by the ratio of stress to strain amplitudes (Fung, 1981, 1984; Ferry, 1961; Wainwright *et al.* 1976).

This ratio, the total dynamic stiffness of the cell, represents the overall resistance to deformation and is called the complex modulus. Because it does not depend upon any phase between stress and strain, it includes both elastic and viscous components of the mechanical behavior of cells. Since stress and strain are not in phase for viscoelastic materials subject to sinusoidal loads, we use two additional moduli: the elastic modulus (E'):

$$E' = E^*\cos\delta \tag{3}$$

and the viscous modulus (E''):

$$E'' = E^*\sin\delta\,, \tag{4}$$

where δ is the phase angle between the maximum stress and maximum strain (Ferry, 1961; Wainwright *et al.* 1976).

Two additional parameters follow directly from the measured moduli and strains. These are the total energy stored (E_s) and the total energy dissipated (E_d) per unit volume of muscle which, for cells that are viscoelastic, are given as:

$$E_s = \int E'\epsilon\,\mathrm{d}\epsilon\,, \tag{5}$$

$$E_d = \int E''\epsilon\,\mathrm{d}\epsilon\,. \tag{6}$$

For linear variations in the mechanical behaviour of cells, these parameters may be approximated as:

$$E_s = E'\epsilon^2/2\,, \tag{7}$$

$$E_d = E''\epsilon^2/2\,. \tag{8}$$

Thus, during escape, the powerful flexor muscles must not only generate sufficient work to propel the animal through its environment but also generate sufficient energy to deform the antagonistic extensor muscles at the appropriate rate. The total energy output for flexor cells during the escape process, therefore, equals or exceeds the sum of the above two terms multiplied by the volume of extensor cells. If the passive viscous component of flexor cells is similar to that of extensor cells, the total energy output, E_{tot}, is given by:

$$E_{tot} = V_{ext}(E_{s,ext} + E_{d,ext}) + V_{flex}E_{d,flex}\,, \tag{9}$$

where V is the volume of a particular muscle group and the subscript s pertains to stored energy, d to dissipated energy, *ext* to extensor cells, and *flex* to flexor cells. This energy output is required only for extending antagonistic muscle groups and overcoming any dissipation of energy in the agonists. It does not include the energy required to accelerate the mass of the animal and to overcome the fluid drag and accelerational forces. Below, we see the extent to which E_{tot}, as defined above, contributes to the total energy required for escape locomotion.

Statistical analysis

To test whether there was a significant peak in our data for phase *versus* frequency we used the join-point method of Hudson (1966) for defining the intersection between two fitted lines. By this method, the data are divided into two subsamples whose sizes are set to yield a maximum regression coefficient with maximum significance.

Results

Physiological range of sarcomere lengths and loading frequencies

As we have previously shown (Daniel and Meyhöfer, 1989), an entire tail-flip lasts about 30–40 ms, with the abdomen moving from a fully extended to a fully flexed position. On the basis of such half-cycle durations, one would predict a loading frequency of about 12–16 Hz. However, two factors confound a direct comparison between such measurements and our sinusoidally imposed length changes applied to single fibers: (i) the tail motion is not exactly sinusoidal, the earlier phases of the cycle occur at greater speeds than the later phases, and (ii) the strains (and strain rates) for *in vitro* cell extensions are about one order of magnitude greater than in our experiments. In short, a 100 Hz loading applied to isolated cells at low strains is nearly equivalent to a 10 Hz tail-flip associated with greater *in vivo* strains. Note, however, that this approximation is an underestimate for the earlier phases of the tail-flip and an overestimate for the later phases.

Electron micrographs of the two cell types (Fig. 2) showed thin filament lengths of 0.87 μm (C1 cells) and 1.0 μm (C2 cells) and thick filament lengths ranging from 1.65–1.8 μm for C1 cells to 1.9–2.0 μm for C2 cells. Therefore, we predict that 100 % filament overlap occurs at 1.9 and 2.1 μm for C1 and C2 cells, respectively. A 10–20 % length change (an estimate from films for maximum abdominal

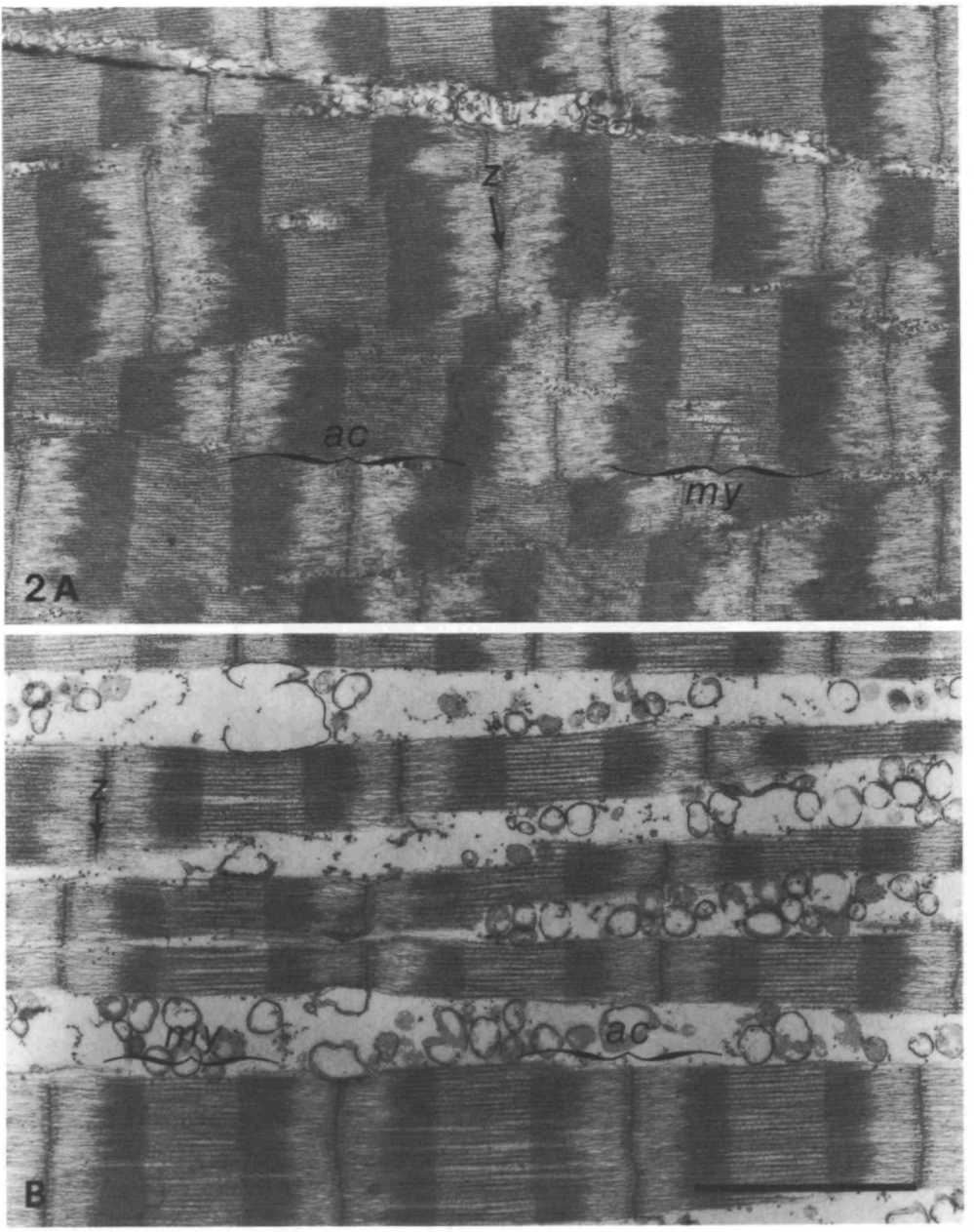

Fig. 2. Transmission electron micrographs of longitudinal sections through two of the cells used in our experiments. (A) C2 cell with a sarcomere length of 3.1 μm. (B) C1 cell with a sarcomere length of 2.7 μm. Both cells were subject to more than 1000 oscillations. *z*, Z-line; *my*, myosin (thick) filaments; *ac*, actin (thin) filaments. Scale bar, 2 μm.

strains) for these cells would lead to sarcomere lengths of 2.3 and 2.5 μm for the C1 and C2 cells, respectively. Our data, therefore, encompass this range but do not include the possible supercontracted state.

Mechanical properties

Our data are summarized by three parameters, the total stiffness (the complex modulus, E^*), the elastic modulus (E′) and the relative viscous dissipation (tanδ). Fig. 3 shows an example of how these parameters vary with frequency for a C1 cell at a sarcomere length of 2.1 μm. An approximately threefold increase in both the complex and elastic moduli was achieved for a change in frequency of more than

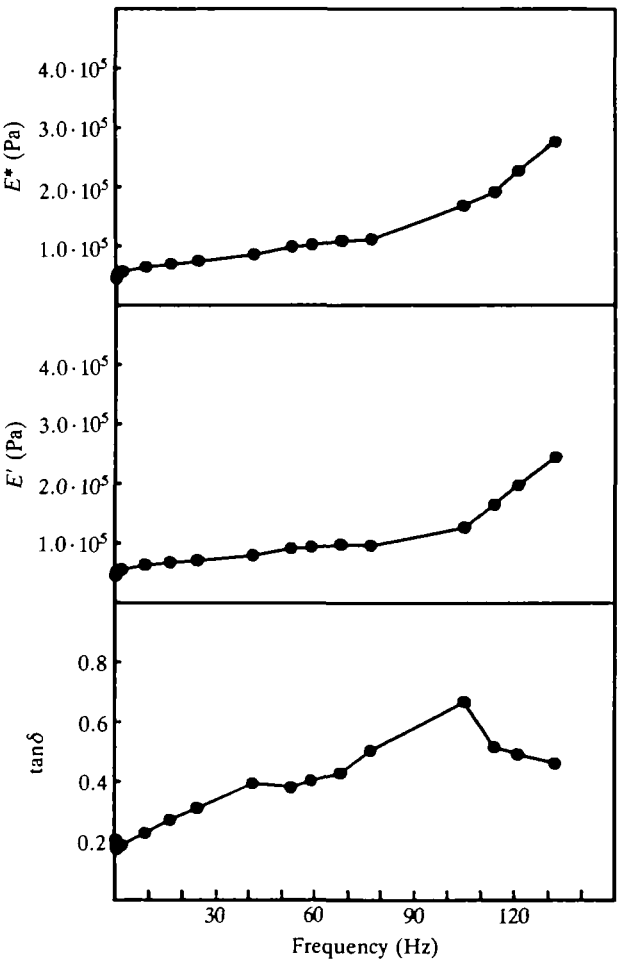

Fig. 3. The complex (E^*) and elastic (E') moduli and the tangent of the phase angle (δ) are plotted as functions of frequency of deformation for a C1 cell whose sarcomere length was 2.1 μm. The increase in these moduli with frequency is quite modest relative to the length dependence of these parameters.

one order of magnitude. Intriguingly, the relative viscous dissipation showed a maximum at about 100 Hz. As is shown below, the sarcomere length has a profound effect on the mechanical properties and we chose, therefore, to represent our combined data in three frequency bins for both cell types (Figs 4 and 5).

Dependence on sarcomere length

Both cell types showed an increase in their overall stiffness (the complex modulus, E^*) and the elastic part of that stiffness (the storage modulus, E') with increasing sarcomere lengths for all frequencies (Fig. 4A,B), a result that is consistent with many previous reports for the non-linear elastic behavior of muscle (Pringle, 1967; Fung, 1984; Tidball and Daniel, 1986). The magnitudes of these parameters for the two cell types were similar, despite a difference in the experimental range in sarcomere lengths. We were unable to extend C1 cells beyond sarcomere lengths of 2.7 μm without breaking them. C2 cells, however, could be extended to 3.2 μm before they broke.

The ratio of viscous to elastic moduli, represented by the tangent of the phase angle between maximum stress and maximum strain ($\tan\delta$), decreased slightly with increasing sarcomere length (lower panels in Fig. 4A,B). Thus, at short sarcomere lengths, cells dissipated a greater proportion of the energy imparted to them than at long sarcomere lengths. Over all sarcomere lengths the ratio of viscous to elastic moduli for both C1 and C2 cells was close to 0.5. This value indicates that a significant proportion of the mechanical energy imparted to extensor cells is viscously dissipated.

Dependence on frequency

Increases in the frequency of loading generally led to increases in both the complex and storage moduli (Fig. 4A,B). This trend appeared to be stronger at longer sarcomere lengths, with the moduli plateauing, or decreasing slightly, at higher frequencies.

The ratios of viscous to elastic moduli ($\tan\delta$) showed intriguing maxima (Fig. 5). For C1 cells at sarcomere lengths between 2.2 and 2.5 μm this peak occurred at about 90 Hz with a value of about 0.5. C2 cells also show a peak in this ratio at similar frequencies. Given that the physiological range of sarcomere lengths is between about 2.0 and 2.4 μm, and that the physiological strain rates correspond to about 100 Hz (see above), these results suggest that extensor cells dissipate mechanical energy maximally during escape locomotion. The existence of a peak in the tangent of the phase angle is statistically significant (F-test on paired regression lines, $P<0.05$).

Discussion

Three intriguing results emerge from our data: (i) increases in either sarcomere length or loading frequency lead to increases in the total dynamic stiffness of the

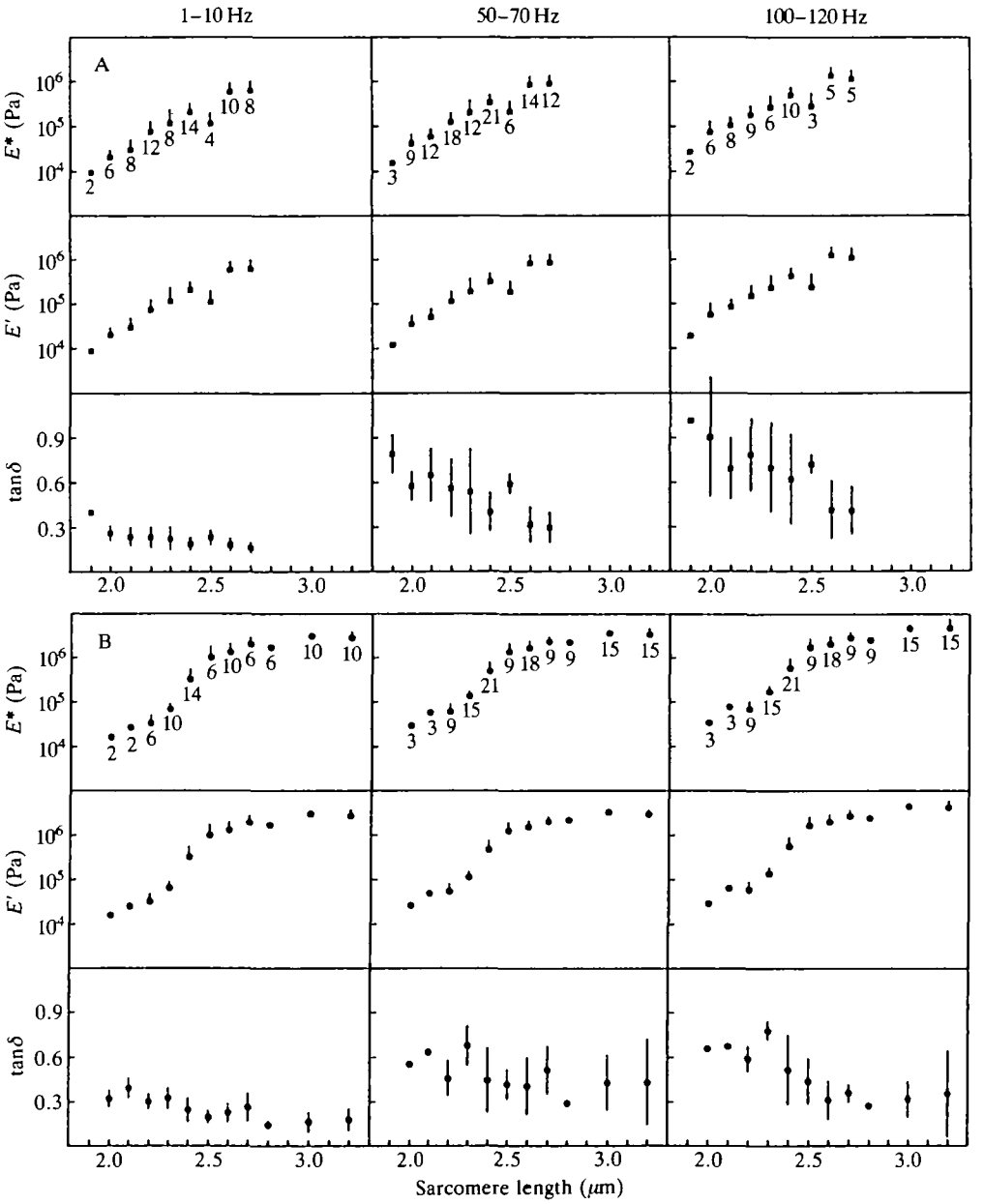

Fig. 4. The complex (E^*) and elastic (E') moduli as well as the tangent of the phase angle (δ) are plotted against sarcomere length for three frequency bins: 1–10, 50–70, and 100–120 Hz and both cell types (C1 cells in A and C2 cells in B). One standard deviation for moduli values is shown as a vertical line extending upwards from the mean. The number of points for each mean is specified beneath each mean. Note the logarithmic scale for the moduli shows that not even a doubling in length leads to a two-decade increase in modulus.

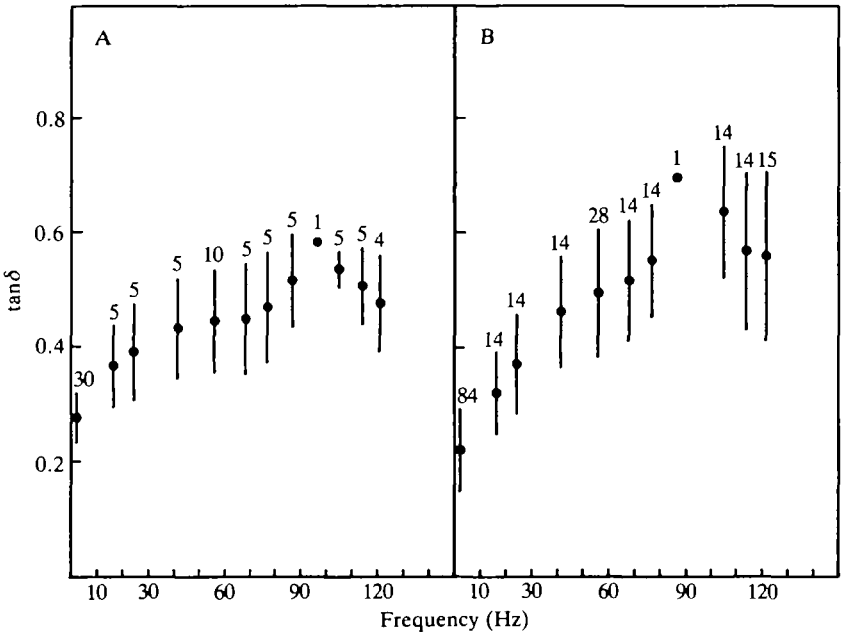

Fig. 5. The mean and standard deviations of the loss tangent (ratio of energy dissipated to energy stored per cycle of oscillation) plotted against frequency for C1 cells (B, sarcomere length range 1.9–2.2 μm) and C2 cells (A, sarcomere length range 2.2–2.4 μm) in bins of frequency of width 10 Hz. The number of values in each frequency bin is specified above each mean.

cell and in the elastic part of that parameter; (ii) the relative dissipation of energy, mediated by the viscous behavior of the cell, is highest in the physiological range of loading frequencies and sarcomere lengths; and (iii) the proportion of energy dissipated in extensor cells constitutes a significant fraction of the total energy required for escape locomotion. Before we discuss the implications of these results, we must first determine whether the magnitudes of the parameters we measured are at all reasonable and whether we may expect to find similar results in muscle cells from other organisms.

Dynamic mechanical properties of muscles in general

In general, data for dynamic mechanical properties of muscle in the relaxed state are few and far between. Indeed, most of the literature focuses almost exclusively on activated muscle fibers with the aim of illuminating cross-bridge mechanisms and rate constants for cross-bridge kinetics. While this may be a logical approach for understanding the basic mechanics of contractility, such studies cannot be used to determine the mechanics and energetics of passive muscle extension. Furthermore, in the absence of basic information about the

dynamic mechanical properties of muscle in the relaxed state, it may be difficult to interpret dynamics of active muscle or to interpret length or tension transients as purely representative of cross-bridge activity. Both viscous and inertial components of muscle cells may affect their response to dynamic loads.

Our study yields values for muscle stiffness that vary over nearly two orders of magnitude depending on sarcomere length and frequency. Are any of these values consistent with previously measured moduli for crustacean muscle in particular or striated muscle in general, and what factors might explain the strong dependence on sarcomere length? Answering these questions by direct comparison with values from the literature is confounded by three factors: (i) the sarcomere length is not always reported; (ii) if dynamic stiffness measurements were made, the frequency may not be in the range of physiological frequencies; and (iii) the state of the preparation (relaxed, activated or rigored) may not be the same as ours. Despite these limitations, however, there are some relevant comparisons. For example, Kawai and Brandt (1980) reported a value of approximately 1 MPa for the complex modulus of relaxed rabbit psoas muscle fibers over a frequency range of 1–100 Hz (unspecified sarcomere length). Their values for crustacean fibers are more enigmatic: the moduli they report are in units of $N m^{-1}$ for activated cells at unspecified sarcomere lengths. While the cell type in their study is presumably quite similar to ours, we can make no direct comparison of moduli owing to the differences in units, physiological state and sarcomere lengths. Studies on the dynamic mechanical properties of other muscle types yield stiffness values that are similar to ours. For example, frog skeletal muscle in rigor has a complex modulus at 1 Hz that increases from 1 to 8 MPa over a sarcomere length range of 2–4 μm (Tidball and Daniel, 1986). These values for cells in rigor are about one order of magnitude higher than ours for relaxed crustacean fibers, although they show the same general trend of an exponential increase in stiffness with increasing sarcomere length. Finally, our relatively high phase angle is consistent with the results of very early studies of viscous damping for muscle fibers in both passive and active states (Buchthal and Rosenfalck, 1957), for which a value of 0.6 for $\tan\delta$ was given.

The exponential rise in both complex and elastic moduli with increasing sarcomere length is quite similar to previously reported trends (Tidball and Daniel, 1986; and data in Hoyle, 1983) and can be attributed, in part, to the presence of an extensive extracellular meshwork of collagen fibers (Tidball, 1986). The basic idea is that these fibers become increasingly aligned with respect to the long axis of the muscle fiber and contribute, therefore, ever greater stiffness with increasing sarcomere length (Tidball and Daniel, 1986).

An increase in the relative viscous modulus ($\tan\delta$), or an apparent peak in that parameter with respect to loading frequency, is a predictable result for some viscoelastic bodies subject to harmonic oscillations (Fung, 1981). For example, a standard viscoelastic element consisting of two springs, with a dashpot in parallel with one spring and in series with the other, yields a distinct peak in $\tan\delta$ (internal friction; Fung, 1981) at one particular frequency. Similar results can be obtained

from more complicated viscoelastic systems that may consist of many elements or may be non-linear (Schmidt and Tondl, 1986). While our study shows that a fairly mild peak in $\tan\delta$ exists, and is not an unreasonable result for viscoelastic systems, we are forced to ask what the physiological consequences of this apparent 'waste' of energy might be, and why it is highest in the physiological range of loading frequencies and sarcomere lengths.

We should also point out that the values for energy dissipation we report may be quite conservative as the level of imposed strain during experiments was kept low (less than 1 %) in order to apply our analyses of the data. In reality, the strains are likely to be significantly higher – by up to 10–20 % – and, at a given frequency, will lead to strain rates near the upper end of the values we imposed. Accordingly, we might expect relative energy dissipation in physiological loading to be nearer the peaks of the values we report.

What are the implications of the viscous behavior for the total energy required for movement?

In analysing the total energy requirements for motility, most previous studies of animal locomotion have neglected the energy imparted to muscle. Those that do incorporate the mechanical work done on extending antagonistic muscle groups usually presume muscle to be elastic, or at least neglect the energy dissipated by the viscous component of its mechanical properties. With our data, we can explicitly examine the consequences of such assumptions for the escape process of carridean shrimp.

From previous work (Daniel and Meyhöfer, 1989; Daniel and Webb, 1987) we know that a shrimp with a body mass of 0.005 kg accelerates at about $100\,m\,s^{-2}$ through a distance of 0.05 m from the time the tail begins its motion to the end of the tail-flip process. These values lead to a rough estimate of 0.025 J for the energy required to accelerate the body. During this time, the hydrodynamic forces (both drag and acceleration reaction) average around 0.5 N, leading to a requirement of an additional 0.025 J for accelerating the mass of the body in water. The total energy required for the escape process is, therefore, estimated to be 0.05 J.

With a cross-sectional area of $10\,mm^2$ for the extensor muscles and an abdomen length of 5 cm, the total energy required to extend the entire volume of this muscle group is estimated from equation 7 to be 0.0125 J. With the loss modulus having a value that is nearly half that of the complex modulus, the total energy dissipated in the extensors is about 0.006 J, or about 10 % of the energy required to accelerate the mass of the body and overcome the fluid forces acting on it.

If the flexor muscles, occupying 75 % of the cross-sectional area of the abdomen, have loss moduli that are similar to those of extensor cells, an additional 0.018 J will be dissipated in this muscle group as well, even during the contractile event. This is consistent with Tidball's (1986) finding that a significant fraction of the loss modulus arises from the sarcolemmal surface coat, a structure presumably unaffected by the state of activity of the cell. Thus, the total energy dissipated by

the viscous component of the mechanical behavior is about 0.024 J, or 50 % of the total energy required to accelerate the mass of the animal and to overcome the fluid forces acting on it during escape.

Why is the relative viscous loss so high?

Such high values for energy dissipation, as well as the apparent peak in energy dissipation in the middle of the physiological range of loading and over the physiological range of sarcomere lengths, begs an important question: why does the viscous behavior represent such a significant fraction of the total energy in escape locomotion? From an adaptive standpoint we would, at first glance, expect the energy dissipated to be small, as the proportion of the total energy devoted to performing mechanical work would logically have to be maximized if selection were to favor rapid escape locomotion.

One possible answer to this question is that it is simply not possible to build biological materials, and especially muscles, which do not dissipate energy. Unfortunately, this pessimistic view is not supported by our data or, for that matter, data from others on a variety of muscle types (Tidball and Daniel, 1986; Pringle, 1967; Kawai and Brandt, 1980; Alexander and Bennet-Clark, 1977; Cavagna *et al.* 1977) that show effective elastic energy storage at some range of lengths or loading frequencies. The data in the present paper, as well as those of previous researchers, suggest that outside the physiological range of sarcomere lengths or loading frequencies, it is possible to have lower loss moduli than in the middle of the physiological range.

We can envisage an alternative interpretation for the significance of high values for the energy dissipation in extensor muscles: energy dissipation may not be 'bad' but, instead, may be required for dynamic stability of rapidly extended cells. For example, any system characterized as having some elasticity and mass, but lacking any viscosity, can respond to dynamic loads with ringing, undamped oscillations, or the production of locally high internal strains (Timoshenko *et al.* 1974). Also, for structures subject to impulsive transients in bending, tension or torsion, there is a corresponding deformation wave that propagates along the length of that structure with a speed proportional to the square root of the elastic modulus (Timoshenko *et al.* 1974). In muscle cells, such waves could potentially lead to very large internal strains. In general, in the absence of any mechanism that would remove energy from such systems, there can be no stable response to continued input of energy. Viscous energy dissipation (damping) provides a means to stabilize such systems or spatially distribute strain when loads are applied impulsively (Timoshenko *et al.* 1974). We suggest that the high viscous energy dissipation in extensor cells of the shrimp is the means by which these animals prevent the development of locally high internal strains in response to the rapidly produced loads in the tail.

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