Pflügers Arch (1992) 420:187–193

Pflügers Archiv
European Journal of Physiology

Shortening velocity and force/pCa relationship in skinned crab muscle fibres of different types

S. Galler and W. Rathmayer

Faculty of Biology, University of Konstanz, P.O. Box 5560, W-7750 Konstanz, Federal Republic of Germany

Received July 17, 1991/Received after revision November 3, 1991/Accepted November 13, 1991

Abstract. Single fibres of three different types, which had been characterized histochemically with regard to differences in myofibrillar adenosine triphosphatase (ATPase) activity and its pH stability, were microdissected from freeze dried preparations of the closer muscle in walking legs of the crab *Eriphia spinifrons*. Shortening velocities were determined in slack tests and under constant load conditions in maximally Ca^{2+}-activated skinned muscle fibres. Force/pCa relationships were also measured for the different types of fibres. Compared with data on vertebrate muscles, all crab muscle fibres required large length changes to reach zero force and showed low Ca^{2+} sensitivity for isometric force generation. The length/time relationship obtained from slack tests had a biphasic course. Maximal velocity of filament sliding differed in the three types of fibres investigated. The filament sliding of type IV fibres was about 3 times faster than that of type I fibres. The values obtained for type II fibres ranged in between. These data are positively correlated with myofibrillar ATPase activity determined histochemically. Ca^{2+} sensitivity of force generation was lowest in the fast type IV fibres. It was high in the slow type I and the faster contracting type II fibres. Ca^{2+} sensitivity in crab muscle seems not to be correlated with speed of shortening.

Key words: Crustacean muscle – Muscle fibre types – Myofibrillar ATPase – Muscle contraction – Shortening velocity – Force/pCa relationship

Introduction

Fibres of vertebrate muscles have been classified into several types on the basis of their contractional properties (e.g. [11, 13, 14]). The velocity of shortening of muscles can be correlated with the myosin adenosine triphosphatase (ATPase) activity of their fibres [3, 6]. It has been shown that histochemically assessed differences in myosin ATPase activity are due to the existence of different isoforms of the myosin heavy chains [25] and that these are responsible for the speed of contraction of different muscle fibres [5, 24].

Most skeletal muscles of crustaceans show a much greater heterogeneity with regard to fibre composition than do vertebrate muscles. The coexistence of several fibre types, classified histochemically on the basis of differences in myosin ATPase activity and in the pH stability of this enzyme, has been reported for a number of different crustacean muscles [10, 15, 20, 23, 28, 29].

As yet, however, there are no studies of the contractile properties of single crustacean muscle fibres of different histochemically assessed types. Contraction responses have been investigated and categorised by determining the speed of isometric force development upon depolarisation of fibres in situ by intracellular current injection [2, 4, 12]. Three forms of contraction were described commonly referred to as slow, fast and intermediate.

The closer muscle in the walking legs of the crab *Eriphia spinifrons* contains four fibre types, termed I to IV on the basis of neuromuscular, histochemical and biochemical properties [15, 16, 22, 23]. According to histochemically assessed differences in ATPase activity, they have been tentatively described [23, 29] as tonic (type I) and phasic (types II – IV). Since the closer muscle permits the microdissection of individual fibres from freeze dried specimens, identified fibres are available for skinned-fibre experiments. Data on shortening velocity and force/pCa relationships of identified crustacean muscle fibres representing different types are reported here for the first time. A short note on some of the results has appeared previously [8].

Materials and methods

Identification and isolation of fibres. All experiments were performed on the closer muscle of the first three pairs of walking legs of the crab *E. spinifrons*. The legs were obtained by inducing autotomy.

Offprint requests to: S. Galler, Zoologisches Institut, Universität Salzburg, Hellbrunnerstrasse 34, A-5020, Salzburg, Austria

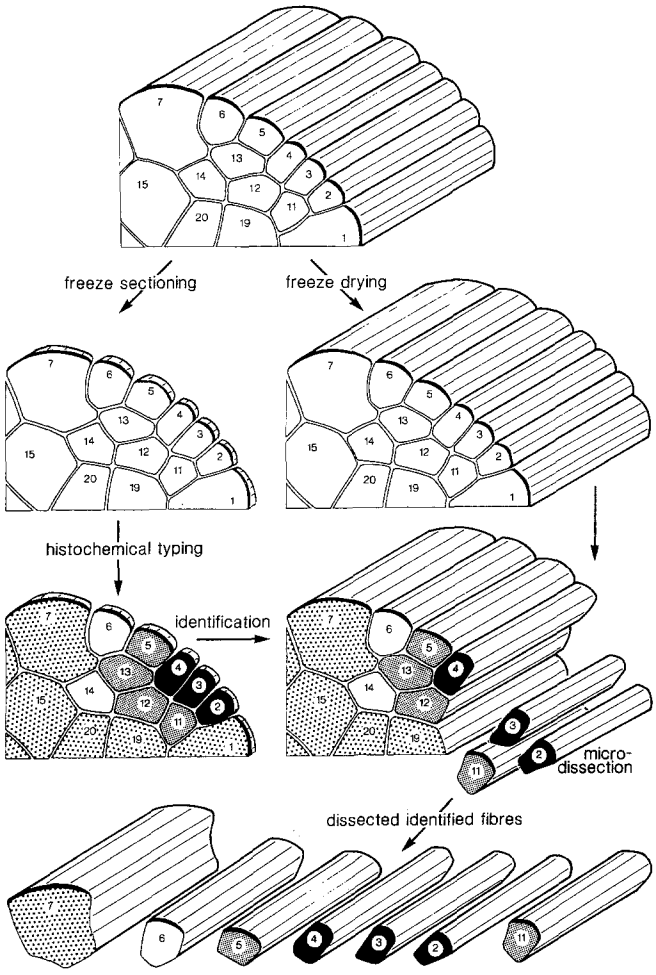

Fig. 1. Experimental procedure to obtain single, histochemically identified muscle fibres for skinned fibre experiments

They were subsequently frozen in isopentane (cooled in liquid N_2) with the dactyl in a stretched position and stored in liquid N_2. The cuticle of the propodite was removed around the closer and opener muscles with a dental drill under liquid N_2. The preparation was then mounted with tissue-tek in a cryostat (−30° C).

The procedure for obtaining identified single muscle fibres of different physiological type is shown in Fig. 1. Starting at the proximal end of the closer muscle, frozen sections of 20 µm thickness were cut until the proximal region of the muscle was reached where all four fibre types are present. To identify the muscle fibres, a few consecutive sections were cut and stained for overall myosin ATPase activity at pH 9.4 and room temperature. Some ATPase determinations were also performed at pH 8.4 and pH 8.7, with the solutions buffered with TAPS [*N*-tris(hydroxymethyl)methyl-3-aminopropanesulphonic acid]. The pH stability of different ATPase isoforms was tested after preincubation of some sections in solutions of different acidity (see [15]).

After cutting a few sections totaling several hundred micrometers for histochemical identification of the fibres, the remaining, major part of the muscle was dried for 4−5 weeks over silica gel at −25° C. To obtain single fibres for mechanical measurements, individual fibres of known histochemical type were microdissected at +20° C and stored individually again at −25° C over silica gel.

Solutions. The bathing solutions were similar to those described previously [17]. Slack tests and most of the force/pCa measurements were performed at 8° C in temperature-controlled vials. The experiments for measuring shortening velocity at different load were performed at 20° C. All solutions contained 60 mM 3-(*N*-morpholino)-2-hydroxypropanesulphonic acid (MOPSO), 8 mM ATP and 10 mM creatine-phosphate. The free Mg^{2+} concentration was calculated to be 1 mM. The solutions for maximal activation contained 50 mM ethylenbis(oxonitrito)tetraacetate, Ca salt (CaEGTA) and 7.3 mM $MgCl_2$, the relaxation solutions 50 mM EGTA and 7.4 mM $MgCl_2$. In the experiments at 20 °C, $MgCl_2$ in the activating solution was 7.4 mM, in the relaxing solution 8.3 mM. The pH of all solutions was adjusted to 6.7 with KOH except those for measurements of shortening velocity at different loads. These had a pH of 7.0; $MgCl_2$ was 7.3 (activating solution) or 8.7 mM (relaxing solution). Unlike the other solutions, they also contained 500 mM sucrose. All solutions contained 15 mM caffeine to eliminate Ca^{2+} release from the sarcoplasmic reticulum. Creatine kinase (20 units per ml) was added to all solutions immediately before the experiments. Ca^{2+} concentrations of the solutions were determined with a Ca^{2+}-sensitive electrode (ETH 129; see also [9]).

Measurement of sarcomere lengths. Single, freeze dried fibres were glued to two glass pins with nitrocellulose dissolved in acetone and then submerged in a relaxing solution (composition as above). Sarcomere lengths were determined before, during and after activation of the fibres with a He-Ne-laser beam (4 mW, 632.8 nm, Spectra Physics, Eugene, OR, USA) measuring the diffraction pattern [32].

Measurements of unloaded shortening velocity (V_{max}). The fibres were glued to the pin of a feedback controlled step motor [maximum velocity (V_{max}) 100 µm in 1.5 ms, 500 µm in 3 ms] and to the peg of a force transducer (AE 801, SensoNor, Horten, Norway) combined with a bridge amplifier (limiting frequency 10 kHz, University of Ulm). Resonance frequency of the transducer with a fibre attached was between 9.5 and 10.5 kHz and was controlled in each experiment. Larger diameter fibres were trimmed with fine glass needles to obtain similar diameters of about 100 µm. Before each experiment the fibres were adjusted to assume just slack position and the sarcomere lengths measured. The length of individual fibres ranged between 2 and 3 mm.

The fibres were activated maximally by quick transfer from a vial containing relaxing solution to one with 10^{-4} M Ca^{2+}. After the isometric force plateau was reached, the motor driven pin was quickly activated. The velocity of release was constant and adjusted to fibre length to yield 1% length change per ms. The fibre slackened and redeveloped force. The time to redevelop force was plotted against the amplitude of slackening. To repeat slack-test experiments with different length changes, the fibres were either stretched again to their original length 1 s after beginning of force redevelopment, or relaxed before restretching by transfer to a low Ca^{2+} solution and then again reactivated maximally by raising Ca^{2+}.

Measurement of shortening velocity at different load. For these experiments the apparatus described above was used with an additional constant load unit (Scientific Instruments, Güth, Heidelberg, FRG), which clamped the actual force by controlling the position of the step motor. After maximal activation of the fibres under isometric conditions at 10^{-4} M Ca^{2+}, the force was clamped in steps of 175 ms duration to four constant values.

The slope of the length changes was determined in the linear portion between 40 and 80 ms after the onset of each force step. Using the Hill equation (see [31]), the unloaded shortening velocity was extrapolated from the measured force/velocity data with a non-linear regression program based on the EUREKA software (Borland, Munich, FRG).

Measurements of force/pCa relationships. The fibres were submerged in relaxing solution (see above) and then activated maximally two or three times in high Ca^{2+}. They were then transferred into several solutions of different Ca^{2+} concentrations and the resulting force was measured.

All statistical values are given with their standard deviation.

Results

Myofibrillar ATPase activity

The fibres of the closer muscle of *E. spinifrons* consist of four types, which can be distinguished histochemically and on the basis of their neuromuscular properties. Type I fibres have low, type II, III and IV fibres high ATPase activity which can be discriminated upon their different pH stability. Using pH 8.4 or 8.7 instead of 9.4 for determination of the overall ATPase activity permits a separation of type II fibres from type III and IV fibres because the staining intensity of type II fibres ranges in between those of type I and III or IV fibres.

Sarcomere lengths

After transferring a fibre into the relaxing solution, the length was adjusted to assume just slack position and sarcomere lengths were measured. Taking the values of all preparations investigated sarcomeres of type I fibres were the longest (14.6 ± 2.5 µm, $n = 56$), those of type IV fibres the shortest (9.6 ± 2.1 µm, $n = 73$). Those of type II fibres ranged in between (12.3 ± 3.0 µm, $n = 58$). However, differences in sarcomere lengths among the different fibre types were not observed in each muscle preparation.

Upon the first maximal activation of the fibres with 10^{-4} M Ca^{2+}, the laser diffraction pattern usually became less distinct. It improved, however, during the subsequent relaxation. When length changes were applied during maximal activation, the pattern deteriorated, but returned after consecutive relaxations of the fibres in Ca^{2+} free medium. The sarcomere lengths determined at the end of each experiment differed not more than 10% from those measured at the beginning for the same fibre length.

Maximal shortening velocities V_{max} (slack tests)

Oscilloscope traces for the determination of the speed of shortening at zero load in a type IV fibre (slack test, [5]) are shown in Fig. 2. The fibre was quickly released after maximal Ca^{2+} activation from the isometric force plateau to shorten and redevelop force at six new lengths (Fig. 2a–f, values given in figure legend). The release slackened the series elastic elements and caused a rapid fall to zero force. The force remained zero as the fibre shortened to take up the slack. The greater the length changes, the longer was the time for redevelopment of force. The onset of force redevelopment (arrows in Fig. 2) at the end of unloaded shortening in each individual slack test was determined from amplified oscilloscope traces.

The length changes required to reach zero force were usually around or more than 10% of fibre length ($n = 56$). These values are considerably larger than the 2% required for slackening in skinned fibres of vertebrate muscles [5, 19].

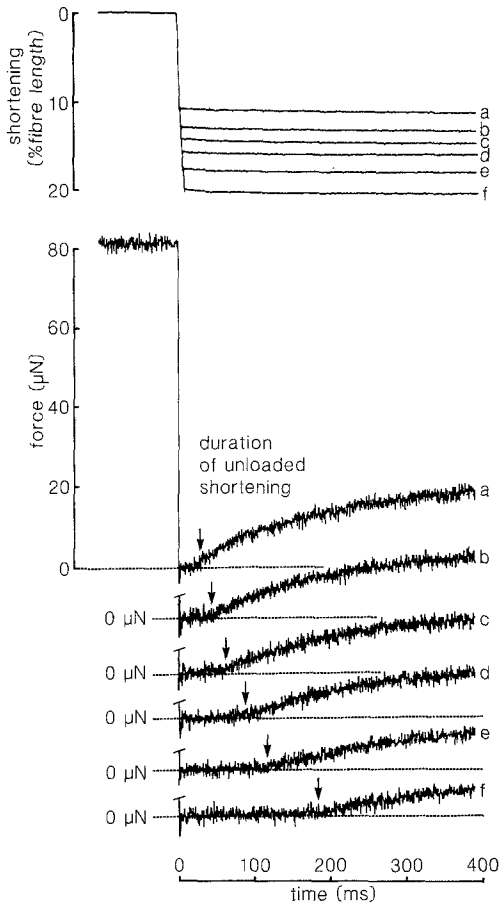

Fig. 2. Original traces of six successive slack tests with a type IV fibre. The fibre was released after maximal Ca^{2+} activation from the isometric force plateau (*top panel*) by six length amounts: *a*, 10.8; *b*, 13; *c*, 14.4; *d*, 15.8; *e*, 17.7; *f*, 20.2 (% fibre length). *Arrows* indicate end of unloaded shortening in each test

The relationship between the time of unloaded shortening from the start of the release to the onset of force redevelopment against the length changes for a type I and a type IV fibre is shown in Fig. 3. The values for each fibre type could be fitted by two linear regression lines, the slopes giving the velocity of unloaded shortening. When calculated for the shorter periods of unloaded shortening (0–200 and 0–400 ms, respectively), shortening velocity was 10.1% fibre length per s (type I) against 76.8% (type IV). For longer periods, the difference was even greater: 1.7% (type I) versus 15.1% (type IV) fibre length per s. Assuming a uniform shortening of all sarcomeres within the muscle fibre, the speed of filament sliding was calculated from the shortening velocity of the whole muscle fibre by multiplication with the half sarcomere length. The values measured for the short periods were 0.54 µm/s per half sarcomere length for the type I against 2.3 µm/s for the type IV fibre.

In 42 out of 53 experiments, the relationship between time of unloaded shortening and length changes had a biphasic course similar to the experiments shown in Fig. 3. The transition point between the two slopes was not constant and there was no obvious dependence on experimental conditions. A biphasic length/time relationship

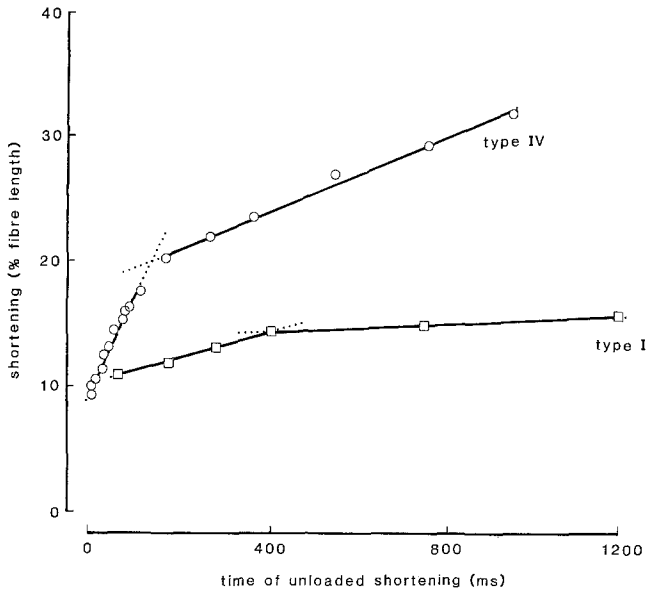

Fig. 3. Comparison of length/time relationships from slack tests employing a type I and a type IV fibre

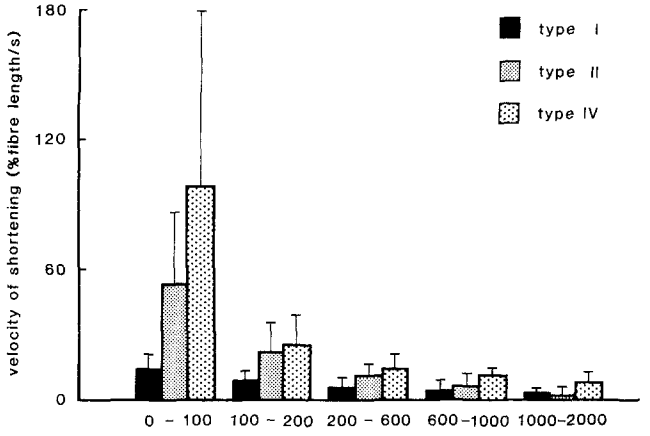

Fig. 4. Means and standard deviation of maximal unloaded shortening velocity of fibres belonging to three different types at different time intervals after onset of slackening

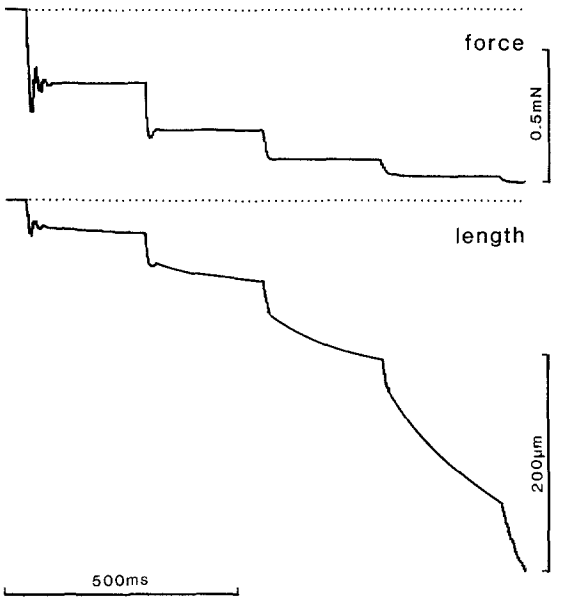

Fig. 5. Original traces of force (*upper panel*) and fibre length (*lower panel*) for four levels of constant load. The beginning of each trace corresponds with the maximally Ca^{2+}-activated isometric force and the respective length of the fibre

was also observed in vertebrate muscle fibres [19], where the effect was explained by the possible existence of two populations of crossbridges with different speed of cycling.

The differences between the fibre types were not always as striking over the whole time scale of unloaded shortening as in the experiments shown in Fig. 3. In order to allow comparison of data from a large number of experiments ($n = 73$), we have divided the time axis into bins of different width (Fig. 4). In experiments with a short period of unloaded shortening (0–600 ms), the shortening velocity of type I fibres was always significantly lower than that of type II and IV fibres ($P < 0.05$). Differences in shortening velocity between type II and IV fibres were present but were not statistically significant. After longer periods of unloaded shortening, the differences between type I fibres and those of the other groups became less pronounced and a statistically significant separation was no longer possible. The velocity of filament sliding calculated from the shortening velocity between 0 and 100 ms and individual sarcomere lengths was 0.86 ± 0.53 µm/s per half sarcomere length ($n = 4$) in type I fibres, 1.63 ± 0.98 µm/s per half sarcomere length ($n = 15$) in type II and 3.05 ± 2.24 ($n = 25$) in type IV fibres. Unfortunately, no data can be presented for type III fibres. Of the 15 type III fibres in the closer muscle, only 4–6 are present in the region of the muscle used in the present investigation [22].

Shortening velocities at different load

An experiment for measuring the shortening velocity of a fibre at different loads is shown in Fig. 5. The upper trace represents force, the lower trace fibre length. The force was decreased stepwise from the isometric force plateau controlled by the constant load unit. In the 5th step, zero force was reached and the fibre slackened.

The changes in fibre length can be separated into two phases. An initial steep phase is followed by a less steep phase (when the force remains stable). The first phase represents the discharge of the series elastic elements of the fibre at the imposed new load, the second the active shortening of the fibre. The time course of shortening was not constant.

Force velocity data for three fibres of different type including those of the experiment shown in Fig. 5 are shown in Fig. 6. The regression lines calculated using the Hill equation (see [31]) are also shown. Taking the results from all experiments together, the extrapolated unloaded shortening expressed as percentage of fibre length per s was 27.8 ± 13.4 ($n = 5$) for type I, 40.2 ± 7.9 ($n = 12$) for type II and 74.4 ± 30.3 ($n = 15$) for type IV fibres.

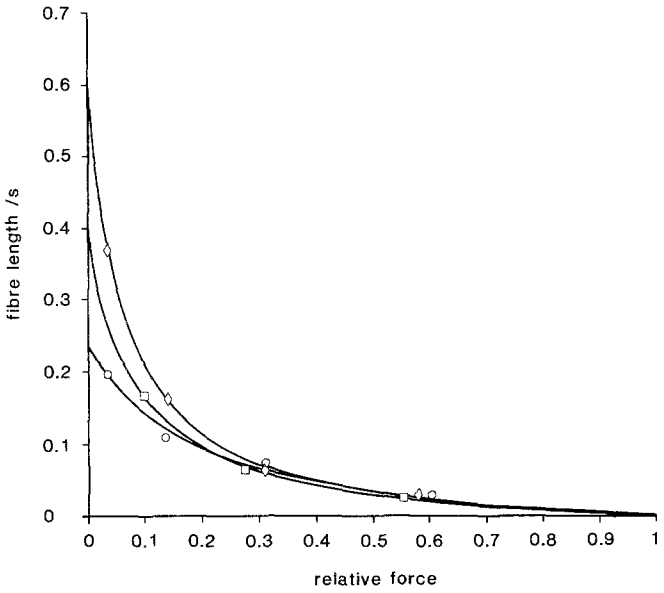

Fig. 6. Force/velocity relationship of three fibres belonging to three different types. (type I = ○; type II = □; type IV = ◇)

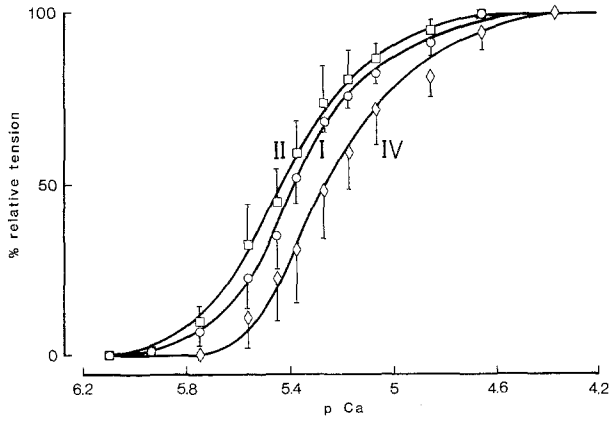

Fig. 7. Force/pCa relationship of fibres belonging to three different types, isolated from the same muscle preparation. Forces were normalized for maximal forces of the individual fibres

Taking into consideration the individual sarcomere length and assuming that sarcomere shortening is proportional to fibre shortening, filament sliding of half sarcomeres was calculated giving values of 1.62 ± 0.71 μm/s for type I, 2.58 ± 0.82 for type II and 4.15 ± 1.8 for type IV fibres. In summary, type I fibres exhibited a filament sliding about one-third slower than type IV fibres. The values for type II fibres ranged in between. This order is in agreement with that obtained from the V_{max} measurements in slack tests.

A direct comparison of the shortening velocities obtained from slack test experiments with those obtained at different levels of constant load is not possible, since experimental conditions (temperature, pH) were different. In addition, when increasing the load in consecutive steps as shown in Fig. 5, shortening velocity is determined not only at different levels of load but also at different states of shortening of the fibres. Load steps to one level only gave higher shortening velocities in comparison with the values obtained when the same load step was applied at the end of a consecutive series of steps (results not shown).

Force/pCa relationship

Steady-state activation curves were obtained for a large number of fibres of different type. From a total of seven muscle preparations, an average of six fibres each of type I, II and IV were analysed for each preparation. The force/pCa relationships for three different fibre types of one muscle preparation are shown in Fig. 7. Relative force (P/P_0) is plotted against pCa. The data is pooled from five type I, six type II and seven type IV fibres. In this preparation, the Ca^{2+} sensitivity was smallest in type IV and greatest in type II fibres.

Considering the data from all muscle preparations (120 fibres), Ca^{2+} sensitivity of force development was generally lowest in type IV fibres ($P < 0.05$). In most of the experiments, it was highest in type II fibres. Differences between type II and I fibres were present, but statistically significant only for the fibres obtained from a single muscle preparation (see Table 1).

The Hill coefficients (slopes of straight lines fitting the experimental data on relative force/pCa in a Hill plot) were greater than two for all three fibre types (for summary see Table 1). In five out of seven muscle preparations (a total of 36 type I, 36 type II and 38 type IV fibres), the Hill coefficient was statistically significant the lowest in the type I fibres. No consistent statistically significant difference could be established between type II and IV fibres.

Discussion

The closer muscle of *E. spinifrons* consists of fibres of four different types [23]. Previously, the mechanical properties of these muscle fibres have not been determined. The type of contraction response was deduced from histochemical determinations of ATPase activity [23, 29]. In the present study, shortening velocity and force/pCa relationship of single identified fibres of different type were determined directly. This closes a gap in the functional understanding of the occurrence of different fibre types in crustacean muscle.

Histochemical staining for ATPase activity at pH 8.4 or 8.7 shows three degrees of staining intensity. Type I fibres have the lowest, type IV fibres the highest activity, type II fibres range in between. The shortening velocity determined from slack tests and constant load experiments and the velocity of filament sliding match closely with the histochemical findings: shortening was fastest in type IV, slowest in type I fibres. The values for type II fibres ranged in between.

Besides the shortening velocity, the total length of the fibres in situ is also of physiological relevance. In the closer muscle of *E. spinifrons*, type IV fibres are generally twice as long as the type I fibres because of different insertion points at the tendon. This factor together with

Table 1. Force/pCa relationship of different types of muscle fibres in *Eriphia spinifrons*. The values for pCa_{50} ($\pm$SD) and the Hill coefficients are given. Experiments 1–5 were performed at 8°C, 6 and 7 at 20°C

Experiment	Parameters for fibre types:					
	type I		type II		type IV	
	pCa_{50}	Hill coefficient	pCa_{50}	Hill coefficient	pCa_{50}	Hill coefficient
1	5.45 ± 0.07 $n = 9$	3.3 ± 0.3 $n = 9$	5.41 ± 0.07 $n = 10$	2.8 ± 0.4 $n = 2$	5.20 ± 0.05 $n = 7$	2.8 ± 0.4 $n = 7$
2	5.38 ± 0.04 $n = 5$	2.5 ± 0.4 $n = 5$	5.44 ± 0.11 $n = 6$	2.7 ± 0.4 $n = 6$	5.25 ± 0.12 $n = 7$	2.6 ± 0.9 $n = 7$
3	5.41 ± 0.03 $n = 2$	2.1 ± 0.2 $n = 2$	5.50 ± 0.05 $n = 2$	2.5 ± 0.4 $n = 4$	5.27 ± 0.05 $n = 4$	2.5 ± 0.4 $n = 4$
4	5.39 ± 0.05 $n = 3$	2.6 ± 0.3 $n = 3$	5.48 ± 0.05 $n = 5$	3.0 ± 0.5 $n = 5$	5.47 ± 0.05 $n = 4$	3.4 ± 0.8 $n = 4$
5	5.42 ± 0.06 $n = 7$	2.4 ± 0.2 $n = 7$	5.40 ± 0.11 $n = 9$	3.9 ± 0.7 $n = 9$	5.28 ± 0.14 $n = 8$	2.9 ± 0.4 $n = 8$
6	5.69 ± 0.10 $n = 6$	2.6 ± 0.6 $n = 6$	5.84 ± 0.07 $n = 6$	3.0 ± 0.5 $n = 6$	5.66 ± 0.10 $n = 8$	4.1 ± 0.4 $n = 8$
7	5.89 ± 0.03 $n = 4$	2.7 ± 0.3 $n = 4$	5.92 ± 0.05 $n = 4$	3.4 ± 0.7 $n = 4$	5.79 ± 0.15 $n = 4$	3.3 ± 0.5 $n = 4$

faster shortening velocity enables type IV fibres to shorten over a several times larger distance per unit time than the type I fibres.

If the velocity of unloaded filament sliding is an indication of the rate of crossbridge detachment [7], the results obtained for *E. spinifrons* imply different relaxation times of the contractile machinery in the different muscle fibre types. In addition, in the living fibres differences in Ca^{2+} sequestration will affect the speed of relaxation. Type I fibres not only possess smaller V_{max}, but should also show slower relaxation than type II and IV fibres.

In vertebrate muscle, Ca^{2+} sensitivity of force generation generally is greater in slow than in fast fibres. The difference, however, varies with the preparation studied: it amounts to 0.3 pCa in the fibres of the fast extensor digitorum longus (EDL) and the slow soleus (SOL) muscle of the rat [26], to 0.7 pCa in fast and slow fibres of the latissimus dorsi muscle of the chicken [18] and to 0.1 pCa in red and white muscles of the dogfish [1]. A similar picture emerged for the type I and IV fibres of *E. spinifrons*. The slowly contracting type I fibres generally had higher Ca^{2+} sensitivity of force generation than the fast type IV fibres. The finding that type II fibres, which shorten 1.5 times faster than type I fibres showed the highest Ca^{2+} sensitivity indicates that in crab muscle this parameter and speed of shortening are not inversely correlated.

A comparison of the Ca^{2+} sensitivity of vertebrate skeletal muscles with that of crab muscle fibres shows lower sensitivity for the latter. However, since force/pCa relationship depends greatly on the parameters of the test solutions, a direct comparison of the values reported in the literature is difficult.

The Hill coefficients for all three types of *E. spinifrons* muscle fibres were greater than 2. In contrast to vertebrate muscle, troponin C isolated from crayfish muscles contains only one Ca^{2+}-binding site [30]. This suggests that cooperativity in crustacean muscle fibres is based on the interaction of separate neighbouring molecules rather than on the cooperativity of several binding sites within the same molecule. A similar situation is assumed to occur in vertebrate muscle fibres [27], although their troponin C contains several Ca^{2+}-binding sites [21]. Since the three fibre types of the closer muscle in *E. spinifrons* exhibit different Hill coefficients, different numbers of Ca^{2+}-binding sites must participate in force generation in the three fibre types present in this muscle.

Acknowledgements. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 156, B5) and the Fond der Chemie. We thank Profs. Kuhn (University of Ulm) and Rüegg (University of Heidelberg) for help, B. Rapp and C. Giele for unfailing technical assistance and B. Cahill for correcting the English version.

References

1. Altringham JD, Johnston IA (1982) The pCa-force and force-velocity characteristics of skinned fibres isolated from fish fast and slow muscles. J Physiol (Lond) 333:159–176
2. Atwood HL, Hoyle G, Smyth T (1965) Mechanical and electrical responses of single innervated crab-muscle fibres. J Physiol (Lond) 180:449–482
3. Barany M (1967) ATPase activity of myosin correlated with speed of muscle shortening. J Gen Physiol 50:197–216
4. Costello WJ, Govind CK (1983) Contractile responses of single fibers in lobster claw closer muscle. Correlation with structure, histochemistry, and innervation. J Exp Zool 227:381–393
5. Edman KAP (1979) The velocity of onloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. J Physiol (Lond) 291:143–159

6. Edman KAP, Reggiani C, Schiaffino S, te Kronnie G (1988) Maximum velocity of shortening related to myosin isoform composition in frog skeletal muscle fibres. J Physiol (Lond) 395:679–694
7. Eisenberg E, Hill TL, Chen Y (1980) Cross-bridge model of muscle contraction. Quantitative analysis. Biophys J 29:195–227
8. Galler S, Rathmayer W (1988) Measurements of contraction-velocity in identified skinned muscle fibers of a crab. Pflügers Arch [Suppl] 411:R191
9. Galler S, Hutzler C, Haller T (1990) Effects of taurine on Ca^{2+}-dependent force development of skinned muscle fibre preparations. J Exp Biol 152:255–264
10. Govind CK, Budd TW, Atwood HL (1981) Fiber composition and innervation patterns of the limb closer muscle in the lobster *Homarus americanus*. Biol Bull 160:69–79
11. Hatcher DD, Luff AR (1985) Force-velocity properties of fast-twitch and slow-twitch muscles of the kitten. J Physiol (Lond) 467:377–385
12. Jahromi SS, Atwood HL (1971) Structural and contractile properties of lobster leg-muscle fibers. J Exp Zool 176:475–486
13. Lännergren J, Smith RS (1966) Types of muscle fibres in toad skeletal muscle. Acta Physiol Scand 68:263–274
14. Lännergren J, Hoh JFY (1984) Myosin isoenzymes in single muscle fibres of *Xenopus laevis*: analysis of five different functional types. Proc R Soc Lond [Biol] 222:401–408
15. Maier L, Rathmayer W, Pette D (1984) pH lability of myosin ATPase permits discrimination of different muscle fiber types in crustaceans. Histochemistry 81:75–77
16. Maier L, Pette D, Rathmayer W (1986) Enzyme activities in single electrophysiologically identified crab muscle fibers. J Physiol (Lond) 371:191–199
17. Moisescu DG, Thieleczek R (1978) Calcium and strontium concentration changes within skinned muscle preparations following a change in the external bathing solution. J Physiol (Lond) 275:241–262
18. Moore G, Johnston IA, Goldspink G (1983) The pCa-tension characteristics of single skinned fibres isolated from the anterior and posterior latissimus dorsi muscles of the chicken. J Exp Biol 105:411–416
19. Moss RL (1986) Effects of shortening velocity of the rabbit skeletal muscle due to variations in the level of thin filament activation. J Physiol (Lond) 377:487–505
20. Ogonowski MM, Lang F (1979) Histochemical evidence for enzyme differences in crustacean fast and slow muscle. J Exp Zool 207:143–151
21. Potter JD, Johnson JD (1982) Troponin. In: Cheung W (ed) Calcium and cell function (vol 2). Academic Press, New York, pp 145–173
22. Rathmayer W, Erxleben C (1984) Identified muscle fibers in a crab. I. Characteristics of excitatory neuromuscular transmission. J Comp Physiol 152:411–420
23. Rathmayer W, Maier L (1987) Muscle fiber types in crabs: studies on single identified muscle fibers. Am Zool 27:1067–1077
24. Reiser PJ, Moss RL, Giulian GG, Graeser ML (1985) Shortening velocity in single fibers from adult rabbit soleus muscles is correlated with myosin heavy chain composition. J Biol Chem 260:9077–9080
25. Staron RS, Pette D (1986) Correlation between myofibrillar ATPase activity and myosin heavy chain composition in rabbit muscle fibers. Histochemistry 86:19–23
26. Stephenson DG, Williams DA (1981) Calcium-activated force responses in fast- and slow-twitch skinned muscle fibres of the rat at different temperatures. J Physiol (Lond) 317:281–302
27. Trueblood CE, Walsh TP, Weber A (1982) Is the steric model of tropomyosin action valid? In: Twarog BM, Levine RJC, Dewey MM (eds) Basic biology of muscles: a comparative approach. Raven, New York, pp 223–241
28. Tse FW, Govind CK, Atwood HL (1981) Diverse fiber composition of swimming muscles in the blue crab, *Callinectes sapidus*. Can J Zool 61:52–59
29. Wiens TJ, Maier L, Rathmayer W (1988) The distribution of the common inhibitory neuron in brachyuran limb musculature. II. Target fibers. J Comp Physiol 163:651–664
30. Wnuk W, Schoechlin M, Stein EA (1984) Regulation of actomyosin ATPase by a single calcium-binding site on troponin C from crayfish. J Biol Chem 259:9017–9023
31. Woledge RC, Curtin NA, Homsher E (1985) Energetic aspects of muscle contraction. Academic Press, London
32. Zite-Ferency F, Rüdel R (1978) A diffractometer using a lateral effect photodiode for the rapid determination of sarcomere length changes in cross-striated muscle. Pflügers Arch 374:97–100