DEPENDENCY OF THE FORCE-VELOCITY RELATIONSHIPS ON Mg ATP IN DIFFERENT TYPES OF MUSCLE FIBERS FROM *XENOPUS LAEVIS*

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ABSTRACT MgATP binding to the actomyosin complex is followed by the dissociation of actin and myosin. The rate of this dissociation process was determined from the relationship between the maximum velocity of shortening and the MgATP concentration. It is shown here that the overall dissociation rate is rather similar in different types of muscle fibers.

The relation between MgATP concentration and the maximum shortening velocity was investigated in fast and slow fibers and bundles of myofibrils of the iliofibularis muscle of *Xenopus laevis* at 4°C from which the sarcolemma was either removed mechanically or made permeable by means of a detergent. A small segment of each fiber was used for a histochemical determination of fiber type. At 5 mM MgATP, the fast fibers had a maximum shortening velocity (V_{max}) of 1.74 ± 0.12 L_o/s (mean ± SEM) (L_o: segment length at a sarcomere length of 2.2 μm). For the slow fibers V_{max} was 0.41 ± 0.15 L_o/s. In both cases, the relationship between V_{max} and the ATP concentration followed the hyperbolic Michaelis-Menten relation. A K_m of 0.56 ± 0.06 mM (mean ± SD) was found for the fast fibers and of 0.16 ± 0.03 mM for the slow fibers. Assuming that V_{max} is mainly determined by the crossbridge detachment rate, the apparent second order dissociation rate for the actomyosin complex in vivo would be $3.8.10^5$ $M^{-1}s^{-1}$ for the fast fibers and $2.9.10^5$ $M^{-1}s^{-1}$ for the slow fibers.

Maximum power output as a function of the MgATP concentration was derived from the force-velocity relationships. At 5 mM MgATP, the maximum power output in fast fibers was (73 ± 8) $mW.g^{-1}$ dry weight and (15 ± 5) $mW.g^{-1}$ in slow fibers. The K_m for MgATP for the maximum power output for the fast fibers was (0.15 ± 0.03) mM, which is about a factor of 4 lower than the K_m for V_{max}. The implications of these results are discussed in terms of a kinetic scheme for crossbridge action.

INTRODUCTION

It is well established that the mechanical performance of muscle during contraction involves a cyclic interaction between actin and myosin, the crossbridge cycle, driven energetically by the hydrolysis of ATP (Huxley, 1957; Infante and Davies, 1962; Lymn and Taylor, 1971; Eisenberg et al., 1980; Woledge et al., 1985; for recent review, see Hibberd and Trentham, 1986). The force-velocity relationship, i.e., the relation between external load and the shortening velocity is a very sensitive index of mechanical and energetic properties. It has been demonstrated for instance that the maximum shortening velocity (V_{max}) which is achieved at zero load, is proportional to the -in vitro- actomyosin ATPase activity (Barany, 1967). This finding was recently confirmed for fibers of different types of the iliofibularis muscle of Xenopus laevis (van der Laarse et al., 1986), by relating V_{max} with the myofibrillar ATPase activity, as determined by a quantitative histochemical method.

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Presently, however, there is no satisfactory explanation for this correlation between mechanical and energetic properties. Maximum shortening velocity at low ATP concentration is probably controlled by the rate of dissociation of actin from myosin (crossbridge detachment) which rapidly takes place after MgATP has bound to the actomyosin complex (Ferenczi et al., 1984). In skinned fibers, from which the membrane has been removed mechanically or chemically, V_{max} depends on the MgATP concentration (Cooke and Bialek, 1979; Ferenczi et al., 1984; Moss and Haworth, 1984). On the other hand, it has been shown that force redevelopment after a release, which is likely to be dominated by the rate of crossbridge attachment, is correlated with the maximum actin activated ATPase activity of myosin crosslinked to actin (Brenner and Eisenberg, 1986). Moreover, Elzinga et al. (1987) found in intact single fibers of Xenopus a linear relationship between the in vivo isometric actomyosin ATPase activity derived from the stable maintenance heat rate and the rate of force redevelopment, while maintenance heat rate and V_{max} were found to be nonlinearly related.

In order to gain more insight in the factors which determine the differences in mechanical and energetical

properties of different types of muscle fibers, we studied the dependency of the force-velocity relationships on the MgATP concentration in different fiber types of the iliofibularis muscle of Xenopus laevis. The iliofibularis muscle of Xenopus laevis contains fibers which can be classified in at least five different fiber types, with large variations in their mechanical and energetical properties (Lännergren, 1978, 1979; Elzinga et al., 1987). Furthermore, Lännergren and Hoh (1984) and Lännergren (1987) have shown differences in the myosin composition in these fibers.

Here we focus on the properties of very fast and very slow fibers from the iliofibularis muscle. Assuming that the kinetic scheme for crossbridge interaction in different types is rather similar (c.f. Taylor, 1979), the results also provide estimates for the rate constants involved in the detachment process and in the processes related to the generation of external work for different fiber types.

METHODS

Bundles containing ~50 fibers were dissected from the iliofibularis muscle of Xenopus laevis. From these bundles, single fibers were isolated in relaxing solution A (Table I). The fast fibers used in this study were obtained from a bundle taken out of the outer zone of the muscle. The slow fibers were isolated from the central part of the tonus bundle. In both cases fibers with a transparent appearance were selected, when viewed with a dissecting microscope under dark field illumination. The rather thick fast fibers from the outer zone used are of type 1, while the thin slow fibers from the tonus bundle are of type 4 or 5, as described by Lännergren and Smith (1966). In isolating fibers, care was taken not to unduly stretch them.

Two different methods were used to render the fibers permeable. In most cases, the fast fibers were mechanically skinned i.e., the sarcolemma was peeled off using sharp needles and forceps, and subsequently split into bundles of myofibrils of ~80 μm in diameter. This had the advantage that from the same fiber several segments could be used. The slow fibers were, in general, chemically skinned by incubating them in relaxing solution A with 0.5% (vol/vol) Triton X-100 (Sigma Chemical Co., St. Louis, MO) for 45 min. The segments were mounted between a thin glass rod extending a force transducer element (natural frequency 2 kHz, Akers AM801, Aksjelskapet Microelectronikk, Horten, Norway) and a displacement servo-system by means of aluminium T-clips, as described by Goldman and Simmons (1984). The fibers could be incubated in different solutions by means of a rotating trough changer. The temperature was kept at ~3°C. Initial sarcomere length, measured by laser diffraction, was adjusted to 2.6 μm. During the measurements, the segments were incubated in relaxing solution (B) for 5 min, pre-activating solution C for 5 min, activating solution D; and from there back again in relaxing solution B. After force had reached its maximum isometric level (P_o), the displacement response to a change in load was recorded. In subsequent activation-relaxation cycles, different relative loads were applied of 0.05, 0.15, 0.25, and 0.5 of P_o. When necessary, corrections were made for a change in baseline of the force transducer which sometimes occurred when the troughs were changed. The solutions contained an ATP regenerating system consisting of 26.2 mM PCr and 0.6 mg/ml creatine kinase (Boehringer, Ingelheim, FRG). An ATP regenerating system similar to this, was shown to be adequate in most frog fibers (Godt, 1974; Ferenczi et al., 1984) which have an ATPase rate similar to fast Xenopus fibers (Elzinga et al., 1987) but a larger diameter.

TABLE I
COMPOSITION OF THE SOLUTION

	EGTA	$CaCl_2$	HDTA	MgATP	PCr
	mM	*mM*	*mM*	*mM*	*mM*
A	20	—	—	5	—
B	20	—	—	0.01 to 5	26.2
C	0.5	—	19.5	0.01 to 5	26.2
D	20	20	—	0.01 to 5	26.2

A dissecting solution, B relaxing solution, C pre-activating solution, D activating solution. In addition each solution contained 60 mM Imidazole, pH = 7.1 adjusted with KOH and 1 mM-free Mg^{2+} and 0.6 mg/ml creatine kinase (200 U/ml at 25°C). Ionic strength was adjusted to 200 mM with KCl. The composition of the solutions was calculated by means of a Fortran computer program using the equilibrium constants listed by Godt and Lindley (1978) at 4°C.

The measurements began at a MgATP concentration of 5 mM. Thereafter the protocol was repeated at a different MgATP concentration, followed by a control measurement at 5 mM MgATP and 0.05 · P_o. The experiment was terminated when isometric force during the control was <80% of the force during the first activation, or when the segment was visibly damaged. In general ~20 activation-relaxation cycles of the same preparation could be used. Each segment could therefore be tested at two or three different MgATP concentrations. After the measurements, the segment was dried in air while still mounted. The dried segment was removed and its length and dry weight were measured by means of a calibrated eyepiece on the dissecting microscope and a Cahn Electrobalance (Cahn 29, Cerritos, Cal. 90701), respectively. Dry weight per unit length was used as a measure of cross-sectional area of the segment. To compare different fibers, force was normalized to dry weight per unit length. The remaining part of each fiber, which was not used for the mechanical experiments, was processed for the determination of fiber type based on myofibrillar ATPase histochemistry (van der Laarse et al., 1986; Stienen et al., 1987*a*). In this method, fiber type is determined from the relation between staining intensity and the ATP concentration in the incubation medium.

Force and length signals were recorded with a pen recorder, a digital oscilloscope, and a computer after A to D conversion at a sample rate of 1 kHz. Shortening velocity was derived from the initial slope of the displacement signal and expressed in segment lengths per second, referred to a sarcomere length of 2.2 μm. The force-velocity relationships were fitted to Hill's hyperbola $(P + a)(v + b) = b(P_o + a)$ in which P is the isotonic load, v is the shortening velocity, and $a/P_o(=\alpha)$ is a measure of the curvature of the relation. The maximum velocity of shortening (V_{max}) at zero load is equal to b/α. The maximum power output (P_{max}) is equal to $bP_o(1 + 2\alpha - 2(\alpha^2 + \alpha)^{1/2})$. V_{max} and P_{max} at the test MgATP concentrations were normalized to the values at 5 mM MgATP concentration and both fitted to a hyperbolic relation through the point of normalization of the form: $y = (1 + K_m/5) \cdot x/(K_m + x)$, in which x is the MgATP concentration and K_m is the MgATP concentration where $y = 0.5 \cdot y_\infty$ (y_∞ is the value at infinite substrate concentration). A nonlinear least squares method (Marquardt, 1963) was used for all curve fittings. For slow fibers especially at 5 mM MgATP, where the force-velocity relationship turned out to be very curved, the hyperbola sometimes had a positive velocity asymptote ($a/P_o < 0$). In these cases, shortening velocities at 0.05 · P_o were used instead of V_{max}, for the determination of the K_m of V_{max} and the maximum power output was obtained from the maximum value of the product of P and V. The results described here, originate from 12 fast fibers of type 1 and 10 slow fibers; 4 fibers of type 4 and 3 fibers of type 5. Due to technical difficulties, the distinction between type 4 and 5 could not be made in 3 slow fibers.

RESULTS

An example of force and length recordings from an experiment on a bundle of myofibrils of a mechanically skinned fast fiber is shown in Fig. 1. Force developed rapidly after the segment was transferred from the pre-

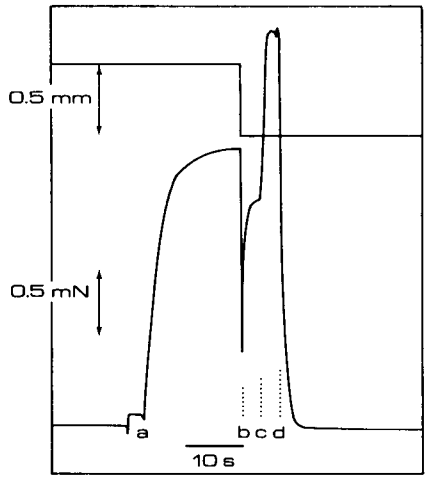

FIGURE 1 Activating-relaxing cycle of a bundle of myofibrils obtained from a mechanically skinned fast muscle fiber at 5 mM MgATP. At *a*, the segment is transferred from the pre-activating solution into the activating solution (pC*a* = 4.4). Force develops in ~10 s and when the maximum isometric level (P_o) is reached, at *b*, the segment shortens at a load of 0.05.P_o until a preset length is reached. From then on force redevelopment takes place. Subsequently, the segment is transferred from the activating solution (at *c*) into the relaxing solution (at *d*). Between *c* and *d* the fiber was kept in air. Experimental conditions: segment length 5.5 mm (at a sarcomere length 2.6 μm), dry weight 0.92 μg/mm, temperature 3.2°C, total length change 0.5 mm.

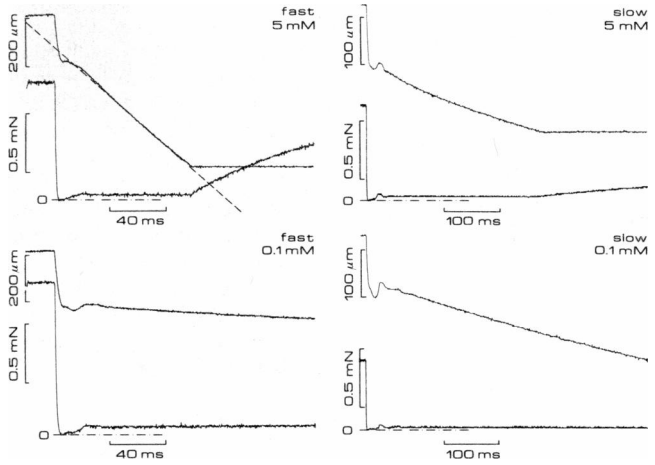

FIGURE 2 Length responses to a load of 0.05.P_o fast fiber (*left column*) and a slow fiber (*right column*), at 5 mM MgATP (*upper row*) and at 0.1 mM MgATP (*lower row*). The shortening velocity was obtained from the initial slope of the length signal, as indicated by the dotted line. In the recordings at 5 mM, the redevelopment of force at the shorter length can be seen. The rate of force redevelopment in the fast fiber is larger than in the slow fiber. At these relatively small loads, the fibers became slack during the initial phase of the shortening, which in combination with the not ideal settings of the feedback loop of the servo-system, gives rise to the oscillations in the early part of the recordings. Experimental conditions: left, see Fig. 1, right; segment length 3.4 mm, dry weight 0.90 μg/mm, temperature 5°C.

activating solution into the activating solution. When the maximum isometric force level (P_o) was reached, an isotonic release was performed, in this example to 0.05 of P_o, and fiber shortening occurred until a preset length was reached. From then on the fiber contracted isometrically again, and a redevelopment of force took place. After a few seconds, the fiber was transferred into relaxing solution. The isometrically developed force as well as the velocity of shortening depended on the MgATP concentration. In Fig. 2 *A*, the response from a fast fiber at 5 mM and 0.1 mM MgATP to a release of 0.05 · P_o are shown together with the corresponding responses obtained from a slow fiber. The period of steady shortening was preceded by an elastic response. In general, series elasticity, which consists of crossbridge elasticity as well as end compliance of the fiber segment, was such that a shortening of ~2% of L_o would reduce force to zero.

At each MgATP concentration force-velocity curves were obtained such as shown in Fig. 3. From the hyperbolic Hill equation which was fitted to the data, values were obtained for V_{max}, a/P_o and maximum power output. In general curvature decreased (increase in a/P_o) at lower MgATP concentrations. At 5 mM MgATP, the curvature of the slow fibers was larger than that of the fast fibers; a finding which is also reported for intact Xenopus fibers at room temperature (Lännergren, 1978, 1979). It can be seen that the reduction in V_{max} at 0.1 mM MgATP relative to the value at 5 mM is larger in fast fibers than in slow fibers at 50 μM. The full dependency of the maximum velocity of shortening on the MgATP concentration is shown for the two fiber types in Fig. 4. To obtain these graphs, V_{max} was normalized for each segment to the value at 5 mM MgATP. The hyperbolic, Michaelis-Menten relation was fitted to the data in order to obtain a value for the K_m as described in the Methods. In this figure, the difference in the K_m value for the fast and slow fibers is clearly seen.

In Fig. 5, the relative isometric force and the relative maximum power output are shown as function of the

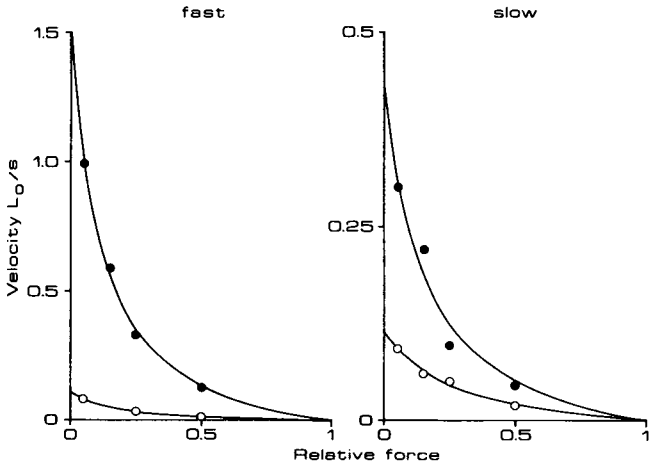

FIGURE 3 Force-velocity relationships at 5 mM and 0.1 mM MgATP for a fast fiber (*left*) and at 5 mM and 50 μM MgATP for a slow fiber (*right*). The continuous lines are Hill curves fitted to the data. Experimental conditions: left; see Fig. 1, right: segment length 2.1 mm, dry weight 0.74 μg/mm, temperature 4°C.

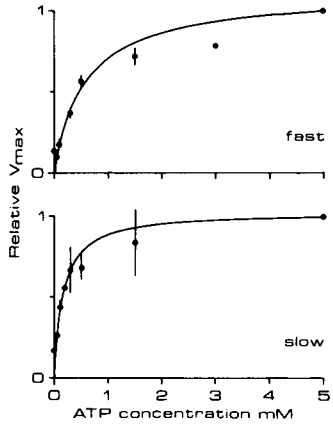

FIGURE 4 Maximum shortening velocity normalized to the value at 5 mM MgATP, as a function of the MgATP concentration for the fast fibers (*upper panel*) and for the slow fibers (*lower panel*). The data (±SEM) are shown. The continuous lines are hyperbola's fitted to the data. Note the difference in the ATP concentration at half maximum V_{max} (K_m) between fast and slow fibers.

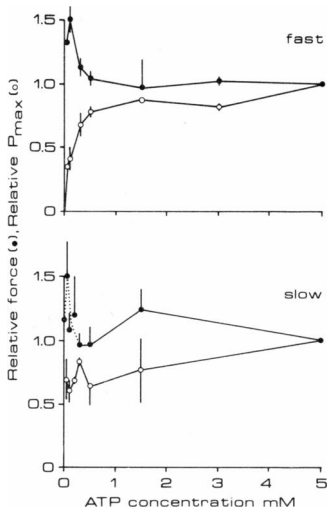

FIGURE 5 Relative isometric force (*filled symbols*) and relative maximum power output (*open symbols*) as a function of the MgATP concentration for fast fibers (*upper panel*) and for the slow fibers (*lower panel*). The SEM is indicated when the error bar was larger than the symbol used, except for the data obtained at the lowest ATP concentration, where, in both cases, only one measurement had been made. The relative force in the fast fibers reached a peak value at 0.1 mM ATP. The K_m for the maximum power was less than the K_m for V_{max} (Fig. 4). For the slow fibers the results are rather variable; as indicated by the dotted line the relative force also shows a peak at low ATP concentration. A reliable value for K_m of the maximum power could not be determined in this case.

MgATP concentration, for fast and slow fibers. In order to correct for deterioration of force development during the experiment, force at lower MgATP concentration was calculated relative to the preceding test measurement at 5 mM MgATP. In fast fibers, relative force increased at lower MgATP concentration, reaching a peak value of 1.5 at ~0.1 mM. In these fibers, maximum power output decreased at lower MgATP concentrations in a hyperbolic fashion; the K_m value is a factor of 4 smaller than the K_m for V_{max}. The results on slow fibers (lower panel, Fig. 5) show scatter. Relative force showed a peak at ~50 μM MgATP. This was, however, more evident in the individual observations than in the averaged results because the concentration at which the maximum was attained varied between fibers. A reliable value for the K_m of the maximum power output in slow fibers could not be determined. This is most likely due to the relatively large variability in V_{max} between fibers. This variability might be due to the different types within the group of slow fibers. Moreover, there is a relatively large uncertainty in the estimates of V_{max} due to the steepness of the force velocity curves. The data suggest a value for K_m of P_{max} below or ~50 μM, which indicates that also for the slow fibers K_m of the maximum power output is considerably smaller than the K_m for V_{max}.

In Table II, a general overview is presented of the quantitative results derived from the whole set of experiments. A statistical analysis, comparing the averaged values with both groups of fibers, yielded significant differences ($P < 0.05$) for V_{max}, K_m of V_{max}, P_{max}, and a/P_o but not for P_o normalized to dry weight per unit length. When the four experiments on slow fibers in which a/P_o at 5 mM MgATP was <0 were excluded, a mean value for a/P_o of 0.101 ± 0.015 was found which was not significantly different from the value obtained for the fast fibers.

DISCUSSION

The average value for the maximum shortening velocity at 5 mM MgATP for the fast fiber preparations was 1.74 L_o/s and 0.41 L_o/s for those obtained from slow fibers. These values were found by extrapolation of the force-velocity curves to zero load. It has been shown that the value for V_{max} is dependent on the extrapolation procedure used (Julian et al., 1986). Their results suggest that V_{max} determined with a slack test would be larger than the V_{max} values presented here. For skinned fibers of Xenopus laevis, Horiuti (1986) presented values for the time required to take up slack, from which values of 2.07 L_o/s for fast fibers (type 1) and of 0.24 L_o/s for slow fibers (type 5) can be calculated. The difference in the V_{max} value in fast fibers of ~20% might well be due to the different

TABLE II
SUMMARY OF THE RESULTS

	V_{max} L_0/s	K_m of V_{max} mM	P_{max} mWg^{-1}	K_m of P_{max} mM	a/P_0	P_0 N.m.g^{-1}
fast	1.74 ± 0.12	0.56 ± 0.06	73 ± 8	0.15 ± 0.03	0.12 ± 0.02	0.78 ± 0.06
slow	0.41 ± 0.15	0.16 ± 0.03	15 ± 5	—	0.06 ± 0.02	0.61 ± 0.07

V_{max}: maximum velocity of shortening in fiber lengths (L_0) per second, K_m: Michaelis-Menten constant for MgATP in mM (± SD), P_{max}: maximum power output in mW per gram dry weight, P_0: isometric force in N normalized to dry weight per unit length (m). a/P_0 indicates the curvature of the force-velocity relationship.
Except for the K_m's, the parameters (±SEM) shown were obtained at 5 mM MgATP.

methods used. However, because of the normalization procedures used here, the influence on the K_m values presented for V_{max} and P_{max} is small. Our value for the shortening velocity of slow fibers is larger than the slack test value for type 5 fibers. This difference could be due to the fact that our group of slow fibers also contained type 4 fibers, which are faster.

The shortening velocity might also be influenced by ADP accumulation and ATP depletion inside the active fibers. These effects depend on the ATPase rate and the diameter of the fibers and therefore they would be larger in fast fibers than in slow fibers. However, by using an ATP regenerating system and by using rather thin bundles of myofibrils of ~80 μm in diameter for the experiments on fast fibers, these effects are likely to be negligible. The rise in the average phosphate concentration during contraction in fast fibers is estimated to be 0.3 mM, indicating that the effects of phosphate accumulation are negligible too.

Lännergren (1978) found in intact fibers of type 2 at 5°C, a V_{max} of 1.49 L_o/s. Taking into account that V_{max} of type 1 fibers probably will be somewhat larger, it follows that the shortening velocity of fast skinned fibers is quite similar to the value in intact fibers. He also found at room temperature an a/P_o value of 0.38 for fast fibers and of 0.10 for the slow fibers and notes that a/P_o was not very sensitive to temperature. Therefore the values for a/P_o and as a consequence also of the maximum power output, are, in skinned fibers, smaller than those found in intact fibers. This is in correspondence with the difference between a/P_o between intact and skinned frog fibers (Ferenczi et al., 1984; Julian et al., 1987). This change in a/P_o by skinning might indicate that the sum of the apparent attachment rate (f) and the detachment rate (g) in skinned fibers is lower than in intact fibers (cf. Simmons and Jewell, 1974). It is well known that upon skinning muscle fibers swell. Reduction of fiber diameters can be achieved by osmotic compression of the fiber. Recently, it was shown by Goldman (1987) that this compression results in a reduction of the shortening velocity at low loads. This indicates that at equal interfilament spacing the maximum shortening velocity in skinned fibers is less than in intact fibers and that the difference in a/P_o between intact and skinned fibers might be related to a difference in interfilament spacing.

Relative force reaches a maximum value of 1.5 at ~50 μM MgATP in fast as well as in slow fibers. In frog a value of 1.2 was found (Ferenczi et al., 1984) while for rabbit psoas fibers a value of ~1.25 was reported (Glyn and Sleep, 1986). In frog, Ferenczi et al. (1984) found for the K_m of V_{max} a value of 0.47 mM, which is close to what we find for fast fibers of Xenopus. Cooke and Pate (1985) report a value of 0.15 mM for rabbit psoas fibers at 10°C. These fibers had a V_{max} of 1.22 L_o/s at 4 mM MgATP. Therefore, the lower K_m values for slower muscle might be a general property of muscle tissue, and as will be shown below, K_m appears to be proportional to V_{max}.

Interpretation of the K_m for ATP of V_{max}

Ferenczi et al. (1984), estimated a value for the apparent second order rate constant for the crossbridge detachment process ($K_1 \cdot k_2$). In their approach, the steps leading to crossbridge detachment are described by scheme 1 (cf. Lymn and Taylor, 1971):

$$AM + ATP \overset{K_1}{\longleftrightarrow} AM \cdot ATP \xrightarrow{k_2} M \cdot \mathrm{ATP} + \mathrm{A}$$

Scheme 1

in which A = Actin, M = Myosin, and AM = Actomyosin complex. A value for k_2 can be calculated from V_{max} at infinite substrate concentration obtained from the Michaelis-Menten relation (fast: 1.95 L_o/s, slow: 0.42 L_o/s), assuming that crossbridges produce force over a distance of 10 nm (Huxley and Simmons, 1971; Simmons and Jewell, 1974). Provided that this assumption also holds for fast and for slow fibers of Xenopus, it follows that k_2 is equal to 215 s^{-1} for fast and 45 s^{-1} for slow fibers. Assuming that the back reaction k_{-1} is small compared with k_2 it follows that K_1 is equal to $1/K_m$. therefore $K_1 \cdot k_2$ is $(3.8 \pm 0.2) \cdot 10^5$ $M^{-1}s^{-1}$ (mean ± SD) for fast fibers and (2.9 ± 0.9) 10^5 $M^{-1}s^{-1}$ for slow fibers. These values do not differ significantly at the 5% level (Student's t test, $P > 0.12$). Our value for fast fibers is somewhat smaller than the value of $5.7 \cdot 10^5$ $M^{-1}s^{-1}$ found by Ferenczi et al. (1984) for frog muscle fibers of the semitendinosus muscle. From an analysis of the slower components in the force response to sinusoidal length changes it was found that the effects of MgATP on the rate constants in fast rabbit muscle occur at larger concentrations than in slow fibers (Kawai and Schachat, 1984) and that the K_m values and dissociation rate constants derived (Kawai, 1982), are rather similar to the values obtained here. Furthermore, it can be noted that for frog myosin subfragment S-1 dissociation in solution a value of $7.4 \cdot 10^5$ $M^{-1}s^{-1}$ was observed (Ferenczi et al., 1978). The $K_1 \cdot k_2$ values for the myosin subfragment S-1 ATPase in solution at low ionic strength of fast posterior latissimus dorsi (PLD) and slow anterior latissimus dorsi (ALD) chicken muscle (Marston and Taylor, 1980), vary in the same proportion as the $K_1 \cdot k_2$ values for fast and slow Xenopus fibers; the quantitative difference between fast and slow skeletal muscle is only ~25%. It can be concluded therefore that the similarity in the $K_1 \cdot k_2$ values for fast and slow skinned fibers also holds for the in vitro determination. It is rather surprising that, although large differences exist in $1/K_1$ and k_2 between fiber types, the product $K_1 \cdot k_2$ is fairly constant. This implies that K_m and V_{max} vary in proportion to each other. However, the in vitro results of Marston and Taylor (1980) suggest that this correlation might hold for skeletal muscle tissue but not for actomyosin from a different source (smooth or cardiac).

Interpretation of K_m for ATP of the Maximum Power Output (P_{max})

The K_m for P_{max} is about a factor of 4 smaller than the K_m for V_{max}. This implies that the power output of a fiber is maintained over a wider range of MgATP concentrations than V_{max}. However, even under rather extreme conditions, like fatigue, the physiological MgATP concentration is not likely to fall below the level where this might become important (Dawson et al., 1980). Its role under normal physiological (working) conditions can, however, not be excluded totally, because the effect of temperature on K_m is not yet known.

The maximum power output is of course influenced by the force and velocity generating capacity of a fiber. This implies that the explanations offered in the literature for the effect of MgATP concentration on force (i.e., the maximum found at low concentration) as well as on shortening velocity (previous paragraph) apply to some extent to the effect of MgATP on the maximum power output as well. The biphasic relation between isometrically developed force and the MgATP concentration, contains two elements; the rise in force at low ATP concentrations up to ~50 μM, in which the cooperative formation of rigor complexes (Brandt et al., 1972; Godt, 1974) as well as actively cycling crossbridges might be involved, and the fall in force, probably due to the dissociating effect of MgATP resulting in a smaller fraction of crossbridge attached at higher MgATP concentrations (Cooke and Bialek, 1979; Ferenczi et al., 1982). This might indicate that at low MgATP concentrations up to ~50 μM, the distribution of crossbridges is shifted towards an attached force generating state. Such a shift in the crossbridge distribution could lead to an increase in force which would then compensate for the decrease in shortening velocity in such a way that the product (power) is maintained high at lower ATP concentrations.

Estimation of the Rate Constants Involved in the Crossbridge Cycle

In order to obtain an insight in the cause of the differences in the mechanical behavior between fiber types, consider the simplified scheme 2 for crossbridge action as proposed by Lymn and Taylor (1971):

$$\begin{array}{ccc} & \overset{1}{\longrightarrow} & \\ \mathrm{AM} & & \mathrm{AM.ATP} \\ {\scriptstyle 4}\uparrow & & \downarrow{\scriptstyle 2} \\ \mathrm{AM.Pr} & \longleftarrow & \mathrm{M.ATP} \\ & \underset{3}{\longleftarrow} & \end{array}$$

Scheme 2

where Pr = ADP.P or ADP; the products of hydrolysis.

The crossbridge attachment rate k_3 can be estimated from the rise time of force development during an isometric tetanus (Lännergren, 1979) assuming a Q_{10} of 1.2, resulting in 20 s^{-1} for the fast and 8 s^{-1} for the slow fibers. Values for k_4 of 1 and 0.1 s^{-1} for the fast and slow fibers, respectively, were obtained from the ATPase rates estimated from measurements of stable maintenance heat (Elzinga et al., 1987; Stienen et al., 1987*b*), assuming a Q_{10} of 4.06 (Curtin et al., 1986). Following the approach of Ferenczi et al. (1984, p. 540) and using the $K_1 \cdot k_2$ values presented above, k_2 = 2,850 s^{-1} and K_2 = 12.5 for fast fibers, while k_2 = 1,450 s^{-1} and K_2 = 5 for slow fibers. The results for fast fibers obtained in this way are very similar to the results on frog of Ferenczi et al. (1984).

If we make the additional assumption that power output is proportional to the net flux of crossbridges undergoing transition 4, which under stationary condition reflects the ATPase rate, it follows that the K_m for ATP of P_{max} is equal to the K_m for ATP of the stationary ATPase rate. In the above scheme this K_m is equal to $k_4/(k_3K_1K_2)$. Therefore k_4 at maximum power output can be derived provided that all other values are equal to the isometric values. This results in k_4 = 7.5 s^{-1} for fast fibers and 0.2 s^{-1} for slow fibers which is then respectively 7.5 and 2 times larger than the isometric turnover rates. The maximum power output (Table II) divided by the crossbridge turnover rate is, in slow fibers considerably larger than in fast fibers. This is compatible with the observation of Woledge (1968) that slow muscle is more efficient than fast muscle.

The presented values for the rate constants have to be interpreted with care because they depend on the crossbridge scheme chosen. Evidence suggests that scheme 2 is not a complete description of the crossbridge cycle during active shortening and that the explanation for the substrate sensitivity should be incorporated into a model in which the transition rates are dependent on the strain of the crossbridges (Eisenberg et al., 1980; Huxley, 1980; Moss and Haworth, 1984). However, the estimated values presented above indicate that, although the overall dissociation rate in fast and slow fibers of Xenopus is rather similar, considerable differences could exist in the individual rate constants.

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