

High mechanical efficiency of the cross-bridge powerstroke in skeletal muscle

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Summary

We were interested to estimate the maximum mechanical efficiency with which chemical energy derived from ATP hydrolysis is converted into mechanical work by individual cross-bridges when they perform their powerstroke synchronously. Glycerinated rabbit psoas muscle fibres, containing ATP molecules almost equal in number to the cross-bridges within the fibre, were activated to shorten under various afterloads by laser-flash photolysis of caged Ca^{2+} . In these conditions, almost all the cross-bridges are in the state where the ATP is hydrolyzed but the products have not yet been released from the cross-bridge (M-ADP-P_i) immediately before activation, and can hydrolyze only one ATP molecule during the flash-induced mechanical response. Power output records of the fibres following activation indicated

that the cross-bridges actually started their powerstroke almost synchronously. The amount of ATP utilized at 1 s after activation was estimated from the amount of isometric force developed after interruption of fibre shortening, while the amount of work done was calculated by multiplying the amount of afterload by the distance of fibre shortening. A conservative estimation of the maximum mechanical efficiency at a load of 0.5–0.6 P_0 was 0.7, suggesting that the actual maximum mechanical efficiency of cross-bridge powerstrokes may be close to unity.

Key words: mechanical efficiency, cross-bridge, skeletal muscle, caged Ca^{2+} , isotonic shortening, muscle work, ATP utilization, rabbit.

Introduction

Muscle contraction is caused by attachment–detachment cycle between the cross-bridges on the thick filament and the thin filament coupled with ATP hydrolysis (A. F. Huxley, 1957; H. E. Huxley, 1960; Bagshaw, 1994). The mechanical efficiency with which chemical energy derived from ATP hydrolysis is converted into mechanical work in demembranated muscle fibres has been estimated recently by measuring the amount of ATP utilized for work production, using fluorescence of a phosphate-binding protein (He et al., 1997, 1999) or NADH (Reggiani et al., 1997; Sun et al., 2001). During myofilament sliding, however, the cross-bridges not only attach to the thin filament to perform their powerstroke-producing positive forces, but also produce negative forces before being detached from the thin filament (A. F. Huxley, 1957). On this basis, the overall mechanical efficiency of muscle fibres may be much smaller than that of individual cross-bridges during their powerstroke, since positive forces are always opposed by negative forces, due to asynchronous cross-bridge activity. To accurately estimate the mechanical efficiency of individual cross-bridges when they perform their powerstroke-producing positive force, it is necessary to perform experiments under conditions in which the cross-bridges start their powerstroke synchronously.

The present work was undertaken to estimate the maximum mechanical efficiency of the cross-bridge powerstroke in

demembranated muscle fibres containing ATP molecules almost equal in number to the cross-bridges (Sugi et al., 1998). The results obtained suggest that the maximum mechanical efficiency of the cross-bridge powerstroke may be close to unity.

Materials and methods

Muscle fibre preparation and experimental setup

Rabbits *Oryctolagus cuniculus* L. were killed by decapitation under pentobarbital anesthesia. Single demembranated muscle fibres (diameter, 40–60 μm ; slack length L_0 , ≤ 2.5 –3 mm), or small bundles consisting of 2–3 muscle fibres, were prepared from glycerinated rabbit psoas muscle (Sugi et al., 1998), and mounted horizontally between a force transducer (AE801, SensoNor, Holten, Norway; resonant frequency, 3.5 kHz; elastic modulus, 2 N mm⁻¹) and a servo-motor (G100PD, General Scanning, Watertown, MA, USA). Fibre cross-sectional area was measured by taking photographs of laterally illuminated fibres (Blinks, 1965). Further details of the method, including the composition of experimental solutions, have been described previously (Sugi et al., 1998). In some experiments, sinusoidal length changes (peak-to-peak amplitude, approx. 0.1% of L_0 , frequency 1–2 kHz) from a waveform generator were applied to

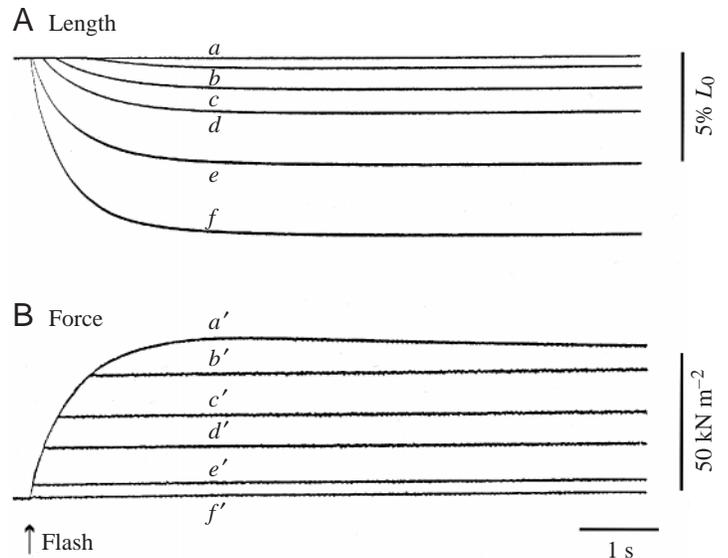


Fig. 1. Laser flash-induced mechanical response. Typical fibre length as a % of L_0 (A) and force (B) changes of the preparation, contracting isometrically (*a*) or shortening isotonicly (*b*–*f*) under five different afterloads. Length recordings *a*, *b*, *c*, *d*, *e* and *f* correspond to force recordings *a'*, *b'*, *c'*, *d'*, *e'* and *f'*, respectively. The loads were P_0 (isometric contraction, *a*, *a'*), $0.78 P_0$ (*b*, *b'*), $0.53 P_0$ (*c*, *c'*), $0.35 P_0$ (*d*, *d'*), $0.09 P_0$ (*e*, *e'*) and 0 (unloaded shortening, *f*, *f'*), respectively. The initial (relaxed) length L_0 and the cross-sectional area of the fibre were 2.8 mm and $6.8 \times 10^{-5} \text{ cm}^2$, respectively.

isometrically contracting fibres to measure in-phase and quadrature stiffness of isometrically contracting fibres by recording resulting force changes (Goldman et al., 1984). The temperature of the solutions was maintained at $1 \pm 0.1^\circ \text{C}$ using a thermoelectric device. The fibres were maximally activated by photolysis of DM-nitrophen (caged Ca^{2+}) with a laser light flash (duration, 8 ns; wavelength, 350 nm; intensity, 20 mJ) from an Nd:YAG laser (DCR3, Spectra Physics). Uniformity of sarcomere spacings along the entire fibre length was confirmed electron microscopically either before or after laser-flash-induced shortening.

Experimental procedures and data analysis

In relaxing solution, the sarcomere length of the fibres was adjusted to $2.4 \mu\text{m}$, at which the overlap between the thick and thin filaments was just maximum (Page and Huxley, 1963). As the extent of fibre shortening was $<15\%$ of the initial fibre length L_0 , the number of cross-bridges interacting with the thin filament was always maximum during fibre shortening. The fibres were kept in prephotolysis solution for 2 min, followed by photolysis solution containing DM-nitrophen for 40–50 s, and were then exposed to air to prevent diffusion of ATP from the photolysis solution that was to be subjected to laser flash irradiation. The ATP concentration of the photolysis solution was determined to be $220 \mu\text{mol l}^{-1}$. A thin layer of photolysis solution at the fibre surface was removed by gently blotting the fibre with a piece of filter paper. Very small rigor force ($\leq 1\%$ of P_0) was always developed in the fibres immediately before flash activation. This indicates that the number of ATP molecules is slightly below, but not above, that of the cross-bridges, since ATP molecules are slowly hydrolysed by the cross-bridges in the relaxed fibres during the time between the moment of exposure of the fibre in air and the moment of laser flash irradiation (2–3 s). The temperature of the space where the fibres were activated was estimated to be 4°C (Sugi et al., 1998).

Length and force changes of the fibres after flash activation

were stored in a digital memory for analysis. To indirectly estimate the amount of ATP (or more exactly M-ADP- P_i , where the ATP is hydrolyzed but the products have not yet been released from the cross-bridge) utilized at 1 s after flash activation (P_u), the fibres were subjected to a quick decrease in fibre length (quick release, 1–2% of L_0 , complete in 1–2 ms) at 1 s after flash activation to drop the force to zero, and then the fibre length was clamped to allow the fibres to develop isometric force. The amount of isometric force developed (P_r , relative to the maximum isometric force P_0) was taken as a measure of the amount of M-ADP- P_i remaining in the fibre at 1 s after activation. The value of P_u was obtained as $P_u = P_0 - P_r$. After a flash-induced mechanical response, the fibres were made to relax in relaxing solution. The flash activation of the fibres could be repeated 5–10 times at 10 min intervals. Data were discarded when the decrease in rate of development of isometric force preceding fibre shortening was recognized.

Results

Characteristics of laser-flash-induced fibre shortening

When the fibres were maximally activated by photo-released Ca^{2+} , they first developed an isometric force equal to the afterload P , started shortening isotonicly, and then eventually stopped shortening as the fibres entered rigor state after complete exhaustion of ATP (Fig. 1). The maximum isometric force P_0 and the unloaded shortening velocity V_{max} were $65 \pm 2 \text{ kN m}^{-2}$ (mean \pm S.E.M., $N=8$) and $0.12 \pm 0.01 L_0 \text{ s}^{-1}$ ($N=8$), respectively. The maximum power output was $0.60 \pm 0.03 \text{ W l}^{-1}$ (where l =fibre volume in litres) ($N=8$). As shown in Fig. 2A, the power output reached a peak at the early phase of fibre shortening, and then decreased with time. The higher the initial peak, the larger the area under the power output trace, i.e. the amount of work done by fibre shortening. The power output records were almost identical when normalized with respect to their peak values (Fig. 2B), except for the load close to P_0 . The distance of fibre shortening when the power output reached a maximum did not

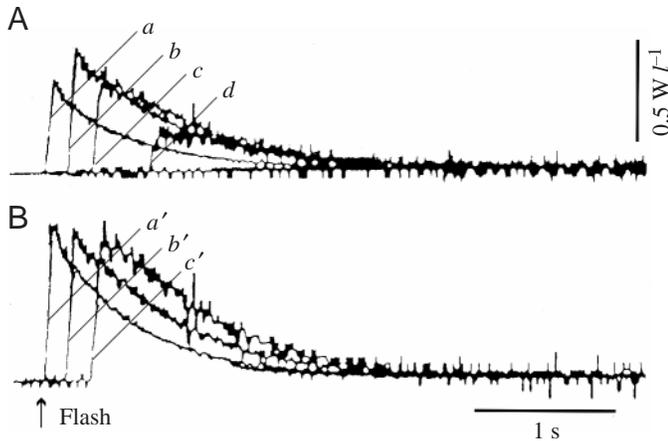


Fig. 2. Power output during flash-induced fibre shortening. (A) Power output recordings under four different afterloads. (B) Power output records normalized relative to the peak values attained. The load was $0.09 P_o$ (*a, a'*), $0.35 P_o$ (*b, b'*), $0.53 P_o$ (*c, c'*) and $0.78 P_o$ (*d*). The recordings were obtained from the experiment shown in Fig. 1.

exceed 10 nm per half sarcomere. This suggests that, at the beginning of fibre shortening, the cross-bridges, in the form of M-ADP-P_i, start their powerstroke almost synchronously, while sensing the amount of load to determine their future energy output.

During isometric contraction, in-phase stiffness, i.e. the magnitude of force changes in response to sinusoidal length changes, increased approximately in parallel with isometric force, while quadrature stiffness, i.e. the 90° out-of-phase stiffness component, reached a maximum at approximately 0.3 s after activation, and stayed almost unchanged for the first

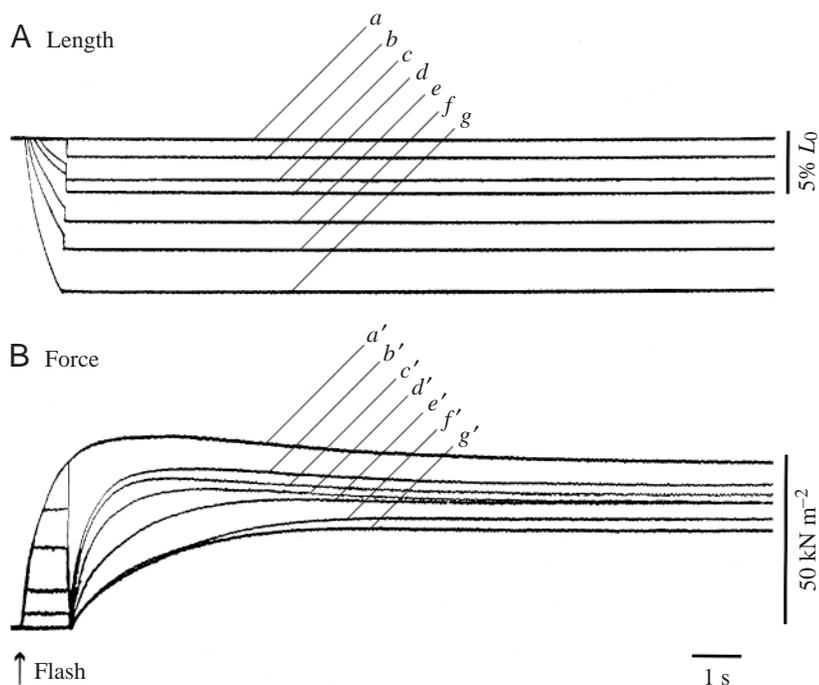


Fig. 3. Fibre length (A) and force (B) changes of a preparation that was first made to shorten isotonicly under five different afterloads, and then subjected to quick releases at 1 s after activation to drop the force to zero. After each release, the preparation redeveloped isometric force at the decreased fibre length. Length recordings *a–g* correspond to force recordings *a'–g'*, respectively. The load was P_o (isometric condition; *b, b'*), $0.63 P_o$ (*c, c'*), $0.41 P_o$ (*d, d'*), $0.20 P_o$ (*e, e'*), $0.09 P_o$ (*f, f'*), $0 P_o$ (unloaded condition; *g, g'*). Recordings *a, a'* were obtained during isometric contraction without quick release.

3–4 s. This indicates that there were no appreciable changes in the number of force-generating cross-bridges during isometric force development preceding fibre shortening, since quadrature stiffness is taken as a measurement of the fraction of active cross-bridges (Goldman et al., 1984). Furthermore, under conditions identical to the present experiments, no appreciable increase of internal resistance against fibre shortening takes place at least for the first 1–2 s after activation (Sugi et al., 1998; Fig. 3). It may therefore be safe to conclude that, at least for 1–2 s after activation, the cross-bridges may not readily form rigor links after releasing P_i and ADP, irrespective of whether the fibre is shortening or kept isometric.

Dependence of the amount of work and the amount of ATP utilized on the isotonic load

Fig. 3 shows a typical experiment in which the fibres were activated to contract isometrically or isotonicly under five different afterloads for 1 s, and then subjected to a quick release to drop the force to zero, whereon the fibre length was clamped and the fibres developed isometric force. The amount of isometric force developed after a quick release (P_T), i.e. a measure of the amount of ATP remaining in the fibre at 1 s after activation, was maximum when $P=P_o$ (isometric contraction) and minimum when $P=0$ (unloaded shortening). Similar results were obtained from 7 different preparations examined. The amount of ATP utilized at 1 s after flash activation ($P_u=P_o-P_T$) was therefore maximum during unloaded shortening ($P=0$), and minimum during isometric contraction ($P=P_o$).

On the other hand, the possibility that cross-bridges forming rigor links with the thin filaments may produce rigor force to contribute to the isometric force development after a quick release can largely be precluded by the extremely slow development of rigor force in glycerinated rabbit psoas fibres (Kobayashi et al., 1998). On application of rigor solution, the ATP concentration at the center of the fibre with radius of 20–30 μm would be reduced to zero within 1 s, if an appropriate diffusion constant of ATP within the fibre ($1.2 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$; Kushmerick and Podolsky, 1969) is taken into consideration. Nevertheless, detectable rigor force development is observed only at 7–10 s after application of rigor

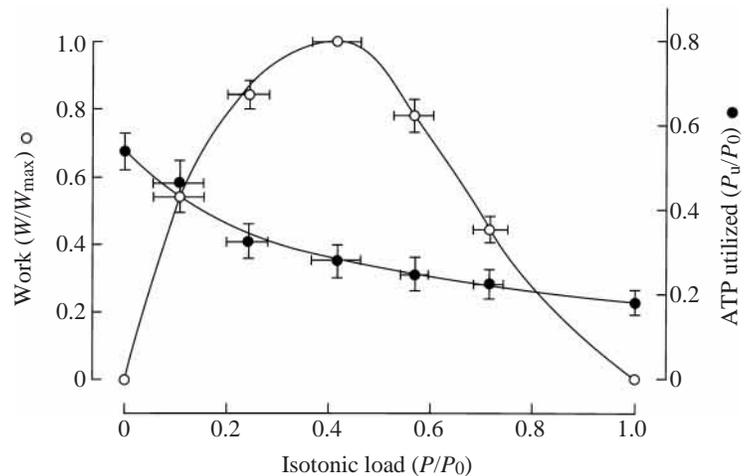


Fig. 4. Dependence of the amount of ATP utilized for mechanical response (P_u , filled circles) and the amount of work done (W , open circles) on the isotonic load (P) at 1 s after flash activation. Values are means \pm S.E.M. obtained from eight different data sets.

solution, indicating a very slow development of rigor force after removal of ATP.

On this basis, the estimation of P_u value may not be influenced by rigor forces, except during isotonic shortening under small loads ($<0.4 P_0$), after which the isometric force development reaches a maximum at more than 7 s after flash activation (Fig. 3). This implies that the value of P_u during isotonic shortening under small loads may be somewhat underestimated, though its extent is very small.

Fig. 4 shows the dependence of the amount of work done (W , expressed relative to the maximum value, W_{max}) and the amount of ATP utilized for the whole mechanical response (P_u , expressed relative to P_0) on the isotonic load (P). The data points were obtained from 8 different data sets. The value of P_u at $P=0$ was approximately 3 times larger than that at $P=P_0$. The value of W was maximum ($1.80 \pm 0.06 \times 10^{-8}$ J, mean \pm S.E.M., $N=8$) at approximately $0.4 P_0$. The W versus P relationship was bell-shaped, since W is necessarily zero at $P=0$ and $P=P_0$.

Dependence of the mechanical efficiency of individual cross-bridges on the isotonic load

The amount of ATP utilized for the whole mechanical response (P_u) is the sum of the amount of ATP utilized for the preceding isometric force development (P_i) and that utilized for the subsequent isotonic shortening (P_s) (see Fig. 7). The value of P_i as a function of isotonic load were obtained by applying a quick release to isometrically contracting fibres at various times after activation and measuring the amount of force developed after each quick release (Fig. 5). Thus, the value of P_s could be obtained by subtracting the value of P_i for a given isometric force equal to the isotonic load from P_u for the whole mechanical response. The value of P_s obtained increased roughly linearly with the distance of fibre shortening, irrespective of the isotonic load (Fig. 6). The mechanical efficiency of individual cross-bridges (E), averaged over the period of work production, can be estimated as $E=W/(P_u-P_i)=W/P_s$, using the results shown in Figs 4 and 5. The dependence of E (expressed relative to the maximum

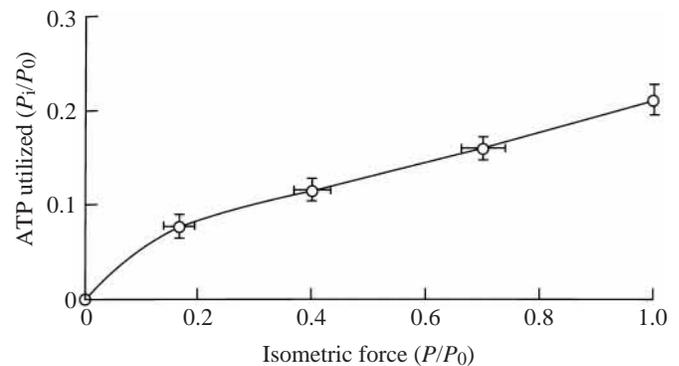


Fig. 5. Relationship between the amount of ATP utilized (P_i) and the isometric force developed (P). Values are means \pm S.E.M. obtained from five different data sets.

value, E_{max}) on the isotonic load is shown schematically in Fig. 7 together with W , P_u , P_i and P_s . The E versus P relationship was bell-shaped, with a broad peak at $0.5-0.6 P_0$.

Estimation of the absolute value of mechanical efficiency of individual cross-bridges

Although the mechanical efficiency of individual cross-bridges is obtained as relative values in the present study, we made a conservative estimation of its absolute value as follows. The average fibre cross-sectional area of 8 preparations, from which the data shown in Fig. 4 were obtained, was $6.1 \pm 0.1 \times 10^{-5}$ cm², while the fibre length was $\leq 2.5-3$ mm. To avoid underestimation of fibre volume leading to overestimation of the efficiency, we use the maximum fibre length of 3 mm to obtain mean fibre volume of 1.8×10^{-5} cm³. Assuming a cross-bridge concentration of $200 \mu\text{mol l}^{-1}$ (higher than the widely used values of 145 or $150 \mu\text{mol l}^{-1}$), the amount of M-ADP-P_i immediately before flash activation is estimated to be $3.6 \times 10^{-6} \mu\text{mol}$ ($200 \times 1.8 \times 10^{-5} \times 10^{-3}$) = 3.6×10^{-12} mol. In Fig. 7, the value of E is maximum at $P=0.53 P_0$, and the corresponding value of P_s is $0.13 P_0$, where P_0 corresponds to the initial amount of M-ADP-P_i of 3.6×10^{-12} mol. The number

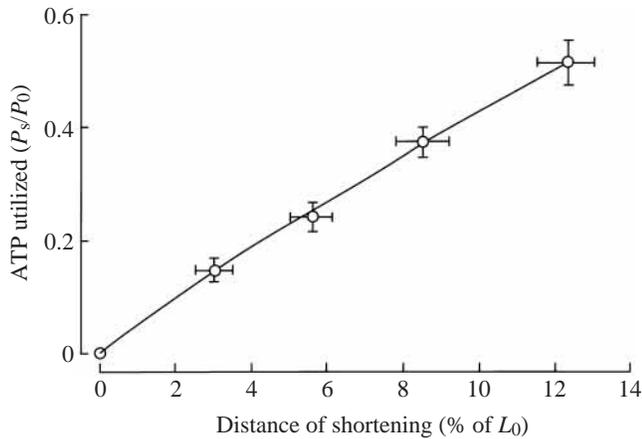


Fig. 6. Dependence of the amount of ATP utilized (P_s) on the distance of fibre shortening. Values are means \pm S.E.M. obtained from eight different data sets.

of ATP molecules utilized for work production is calculated to be 2.8×10^{11} ($3.6 \times 10^{-12} \times 0.13 \times 6 \times 10^{23}$). Assuming the energy released by ATP hydrolysis of 50 kJ mol^{-1} (Bagshaw, 1994; Oiwa et al., 1991), the energy available from one ATP molecule is $8.3 \times 10^{-20} \text{ J}$ ($50 \times 10^3 / 6 \times 10^{23}$). The energy released from ATP molecules during work production is $2.3 \times 10^{-8} \text{ J}$ ($2.8 \times 10^{11} \times 8.3 \times 10^{-20}$). In Fig. 7, the amount of work done at $0.53 P_0$ is $1.6 \times 10^{-8} \text{ J}$. The maximum mechanical efficiency of individual cross-bridges is therefore estimated to be $(1.6 \times 10^{-8}) / (2.3 \times 10^{-8}) = 0.7$. Since the above estimation is conservative, the actual maximum mechanical efficiency of an individual cross-bridge is suggested to be 0.8–0.9, which is close to unity.

Discussion

Validity of the present work to estimate the mechanical efficiency of individual cross-bridges

The aim of the present work was to estimate the mechanical efficiency of individual cross-bridges when they start their powerstroke synchronously. As the number of ATP molecules in the fibre is made almost equal to that of cross-bridges, all

the cross-bridges immediately before activation are in the state M-ADP- P_i , in which ATP is already hydrolyzed but the products ADP and P_i are still bound to the cross-bridge. On laser flash activation, the cross-bridges sequentially release P_i and ADP to build up a flash-induced mechanical response, but after the product release cross-bridges can no longer hydrolyze ATP molecules. This experimental condition may be comparable with that of quenched flow experiments, in which enzyme concentration is equal to substrate concentration, resulting in a single turnover for each enzyme molecule. Since the cross-bridges do not form appreciable rigor links with the thin filament until 1–2 s after activation, it is possible to measure the amount of work done and ATP utilized without any appreciable internal resistance.

At the beginning of fibre shortening, the power output rose rapidly to a peak, and then decreased with time (Fig. 2). The distance of fibre shortening at the peak of power output was $<10 \text{ nm}$ per half sarcomere. This can be taken as evidence that, at the beginning of fibre shortening, the cross-bridges start their powerstroke almost synchronously. In the present study, the period of fibre shortening was restricted to be $<1 \text{ s}$ (Fig. 3). Since the maximum rate of ATP utilization was 0.80 s^{-1} per cross-bridge during unloaded shortening (Fig. 4), the average duration of ATP hydrolysis cycle was 1.3 s, and this value increased up to approximately 5 s with increasing load towards P_0 . This implies that, under large loads, a considerable fraction of cross-bridges, starting their powerstroke at the beginning of fibre shortening, would continue their ATP hydrolysis cycle over the whole period of work production. The mechanical efficiency obtained in the present study may therefore be regarded as largely reflecting that of individual cross-bridges, especially with large loads.

For the reasons stated above, the present results may constitute evidence that the maximum mechanical efficiency of individual cross-bridges may be very high, probably close to unity (0.8–0.9). In this connection, it is of interest to note that it has also been suggested that the mechanical efficiency of the ATP-dependent rotary motion of F_0 - F_1 ATPase at the mitochondrial membrane is close to unity (Kinosita et al., 2000).

Relationship with previous studies

Due to the limited amount of ATP in the fibre and the low temperature at which the present experiments were done, the maximum power output of the fibres in the present study (0.6 W l^{-1}) was more than one order of magnitude smaller than the value obtained from

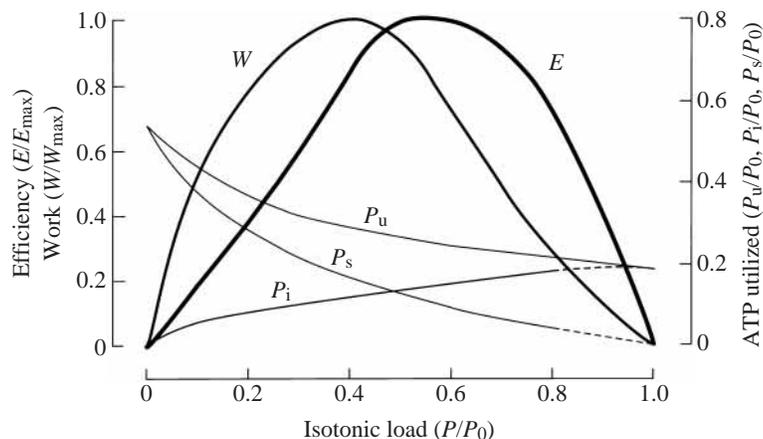


Fig. 7. Dependence of the mechanical efficiency of individual cross-bridges (E) on the amount of isotonic load (P) obtained from the results shown in Figs 4 and 5. Values are scaled to adjust the value at $1.0 P/P_0$ to that in Fig. 4. The amount of work done (W), ATP utilized for whole mechanical response (P_u), ATP utilized for preceding isometric force development (P_i), and ATP utilized for isotonic shortening (P_s) are also shown as functions of load.

rabbit psoas fibres (28 W l^{-1} at 12°C) (He et al., 1997) even when the high Q_{10} value (>5) (He et al., 2000) is taken into consideration. The maximum mean rate of ATP utilization for the first 1 s after activation (0.80 s^{-1} per cross-bridge, Fig. 4) was also markedly smaller than the value of 18.5 s^{-1} per cross-bridge (at 12°C) (He et al., 1997). Meanwhile the amount of ATP utilized for the first 1 s after activation (P_u) increased with decreasing load P , reaching a maximum at $P=0$ without any sign of leveling off (Fig. 4). This may be consistent with the result that a roughly proportional relationship exists between the rate of ATP utilization and the fibre shortening velocity (Reggiani et al., 1997; He et al., 2000; Potma and Stienen, 1996), but not with the biphasic relationship between the rate of energy liberation (heat + work) and the shortening velocity in whole muscle (Hill, 1964; Linari and Woledge, 1995). The approximately linear dependence of the amount of ATP utilized for work production on the distance of shortening (Fig. 6) has already been reported by Sun et al. (2001), suggesting that, irrespective of whether the amounts of ATP available for the cross-bridges are limited or not, the amount of ATP hydrolyzed is primarily determined by the distance of fibre shortening.

The maximum mechanical efficiency of Ca^{2+} -activated skeletal muscle fibres has been reported to range from 0.2 to 0.46 (He et al., 1997; Reggiani et al., 1997; Sun et al., 2001), indicating that the net maximum mechanical efficiency of cross-bridges during their asynchronous activity is much smaller than the maximum mechanical efficiency of individual cross-bridges obtained in the present study. In this connection, it is of interest that, in demembranated cardiac myocytes, the maximum Ca^{2+} -activated isometric force increases by one third when the ATP concentration is reduced to $200 \mu\text{mol l}^{-1}$ (Fabiato and Fabiato, 1975). This might result from an increased degree of synchronization of force-generating cross-bridge activity, when the ATP concentration is reduced to be nearly equal to that of cross-bridges.

The present experiments are closely related to those of Oiwa et al. (1991), who measured the amount of work done by ATP-induced sliding of a myosin-coated microneedle along actin cables in giant algal cells. In response to a limited amount of iontophoretically applied ATP, myosin molecules on the needle moved along actin cables by bending the needle for a distance. By application of ATP under various baseline forces generated by the bent needle, they obtained a bell-shaped work *versus* baseline force relationship similar to the present E *versus* P relationship (Fig. 7), both exhibiting a peak at approximately $0.5 P_0$. This seems to indicate that, irrespective of whether the cross-bridges are regularly arranged in the fibres or randomly oriented on the needle, they sense the amount of load and determine their future work output when they are allowed to produce work. The mechanism underlying the load-dependent mechanical

efficiency of individual cross-bridges remains to be investigated, although it is suggested that their nucleotide affinity changes depending on the strain in the cross-bridge structure (Geeves and Holmes, 1999).

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