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STRIATED SCALLOP MUSCLE RELAXATION: FAST FORCE TRANSIENTS PRODUCED BY PHOTOLYSIS OF DIAZO-2

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Relaxation of the myosin regulated striated adductor muscles of *Pecten maximus* was initiated by the photolysis of the caged Ca^{2+} chelator, Diazo-2. The fibres relaxed to approximately 30% of the maximum tension with a mean half-time of 17.9±1.6 ms (n=7, temp 12°C), much faster than the rates observed in intact muscle at the same temperature. This indicates that in the intact adductor muscle the slower relaxation rate is determined by the speed of Ca^{2+} removal from the sarcoplasm. The faster rate of relaxation of scallop muscle *in vitro*, compared with frog skeletal muscle may reflect different mechanisms of regulation of the crossbridge cycle.

In molluscan striated muscle the Ca^{2+} sensitivity is conferred by a myosin light chain (1), and force generation is inhibited by the configuration of this regulatory light chain in the absence of Ca^{2+}. The loss of the regulatory myosin light chain leads to a loss of sensitivity to Ca^{2+} (2) a high ATPase activity and force. Thus regulation is described as myosin-regulated. This is in marked contrast to vertebrate striated muscle in which troponin-C, located in the thin actin filaments, acts as the Ca^{2+} sensor (3) and together with tropomyosin inhibits a high force generating state of the crossbridge.

Contraction in molluscan striated muscle has been investigated in intact (4) and skinned fibres (5), and found in both cases to be of a similar rate. Studies of relaxation of intact adductor muscle fibres have shown the half time of relaxation to be 104±2 ms. However, to date, there has been no study of the process of relaxation of skinned molluscan striated muscle fibres.

Diazo-2 is a photolabile chelator which can be used to study muscle fibre relaxation in skinned fibres (6) by the rapid removal of Ca^{2+}. Diazo-2 is a derivative of BAPTA (7), which upon photolysis

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rapidly (>2000 s^{-1}) converts from a chelator of low Ca^{2+} affinity (K_d=2.2 μM) to a high affinity chelator (K_d=0.073 μM). The speed of the Ca^{2+} transient using this technique overcomes the problems found using previous techniques caused by the slow rate of diffusion. Diazo-2 has been used successfully to study relaxation in single frog fibres (6).

We have investigated the rate of relaxation of striated adductor muscle of the scallop using the technique of laser flash photolysis of Diazo-2, applied to skinned fibres (5,6).

METHODS

Small bundles of striated adductor muscle fibres (diameter 70-210 μm) were dissected from the giant scallop *Pecten maximus* in a relaxing solution at 12°C, using a similar method to that described by Simmons and Szent-Györgyi (7). T-shaped aluminium foil clips were attached to the ends of a segment of fibre (approx. 3mm). The fibre was suspended between two stainless steel hooks, one attached to a tension transducer (Akers) and the other fixed. The fibres were chemically skinned by immersion in a relaxing solution containing 1% (v/v) Triton X-100 for 20 minutes at 12°C. The apparatus for the photolysis technique and the recording of the force transients was similar to that described previously by Ferenczi et al (8). A frequency-doubled ruby laser (Lumonics, Rugby, England) produced 20 ns pulses of light at 347 nm. The energy of the beam could be controlled by altering the charging potential of the capacitors which drove the xenon flash lamps of the laser. The laser beam was focused using a cylindrical lens and shuttered off so that the area of illumination could be controlled.

After skinning was complete the fibres were placed in a relaxing solution, the sarcomere length was adjusted to 2.3 μM and the dimensions of the fibre were measured. The fibres were then transferred to the Diazo-2 solution, via a relaxing solution which contained no Ca^{2+} buffer, and the Ca^{2+} allowed to equilibrate. The level of free Ca^{2+} in the Diazo-2 had previously been adjusted to produce a maximal contraction prior to illumination and a large relaxation upon flashing. After the Ca^{2+} had reached equilibrium, the trough containing the Diazo-2 was lowered pneumatically from the fibre, suspending the fibre in air. After 600 ms the laser flashed and the fibre was allowed to reach a steady state before being placed in a trough of relaxing solution. By keeping the fibre at a temperature of 12°C, which is the same temperature as the dew point (9), the temperature of the fibre remained constant.

Diazo-2 was made up in a stock solution to a concentration of 2 mM. The stock solution contained 60 mM Tes, 5.0 mM ATP, 5.0 mM Mg^{2+} and 10 mM creatine phosphate. Potassium propionate was added to give the solution an ionic strength of 0.21 M and the pH was adjusted to 7.00. The relaxing and activating solutions were prepared with 30 mM EGTA and 10 mM CaEGTA respectively in the basic stock solutions and was adjusted to the same pH and ionic strength as the Diazo-2 solution.

The output from the force transducer was recorded simultaneously upon a pen recorder (Pantos) and an oscilloscope (Nicolet).

RESULTS

Fig.1 shows the tension recording from a small bundle of skinned fibres upon photolysis of 2 mM Diazo-2. The fibres were initially in a relaxing solution containing 30 mM EGTA. From this trough they were transferred to a relaxing solution with no Ca^{2+} buffer and after a short time transferred to the Diazo-2 solution. The fibres were left in the Diazo-2 solution until the Ca^{2+} binding reached equilibrium. Shortly after this steady state was reached the trough was dropped and 600 ms later the fibre was illuminated by a 75 mJ pulse of light (347 nm, 20 ns duration). The photolysis of Diazo-2 produced an immediate relaxation by the fibre. After 5-10 seconds the fibres were transferred to the trough containing relaxing solution. When the force had returned to a baseline level the fibre was transferred first to a relaxing solution with no Ca^{2+} buffer and then to an activating solution (pCa 4.5). The maximal force produced by the activating solution was the same as the force recorded prior to the laser flash, demonstrating that the contraction in Diazo-2 was indeed near maximal.

Upon flashing the tension declined with a mean half time of 17.9±1.6 ms (n=7) to a mean tension level of 30.4% of the maximum tension. This is significantly faster than the relaxation rates observed in intact fibres, and is in contrast to the results from frog skeletal muscle in which the half times of relaxation in skinned and intact fibres were similar.

DISCUSSION

We have been able to produce a very rapid fall in the free Ca^{2+} concentration within skinned striated adductor muscle fibres, by using the technique of laser-flash photolysis of the caged chelator, Diazo-2. This has enabled us to study the rate of relaxation of the fibres from a maximal contraction, and compare this with previous results from skinned frog striated muscle (4,10)

Using laser flash photolysis of Diazo-2 we found the rate of relaxation of scallop fibres to be extremely fast ($t_{\frac{1}{2}}$ 17.9±1.6 ms), considerably faster than the observed rates in intact fibres ($t_{\frac{1}{2}}$ 104±2 ms) (4). In intact adductor fibres the sarcoplasmic reticulum

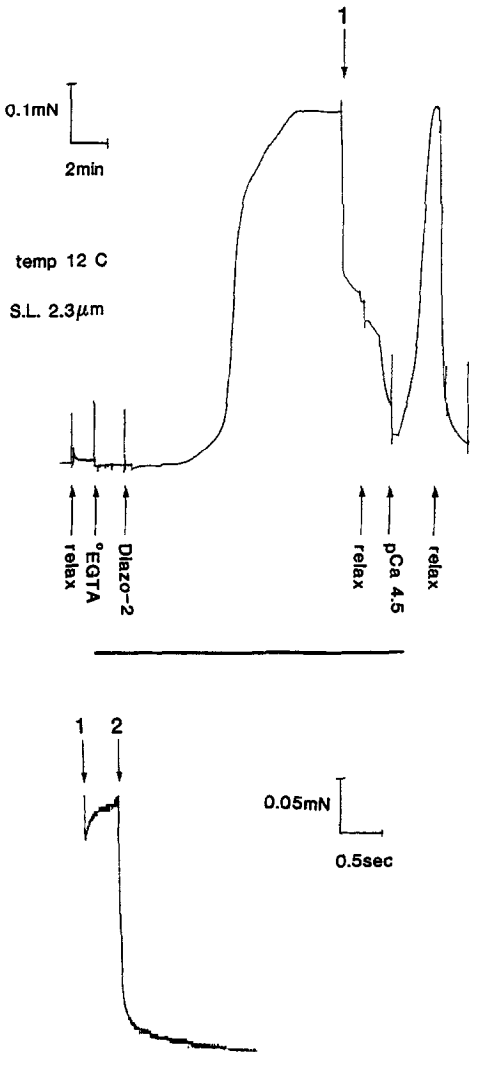


Fig.1 Above: The tension record from skinned myofibrils which were allowed to come to equilibrium in Diazo-2 and then flashed in air with a pulse of light (347 nm) from a frequency-doubled ruby laser. 1 - trough lowered and laser flash. This is followed by a contraction in activating solution (pCa 4.5) showing that the contraction in Diazo-2 was maximal. Just prior to flashing the speed of the chart recorder was increased 10 times. Diam. 209 μm, length 3.0 mm, $t_{\frac{1}{2}}$=14 ms
Below: The same record shown on a faster time scale. 1 - trough lowered. 2 - laser flash.

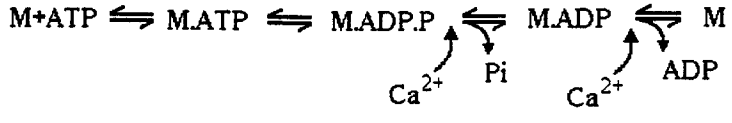
is responsible for the uptake of Ca^{2+} upon relaxation; however, in Triton X-100 skinned fibres this system is non-functional. The fast rates of relaxation from this study demonstrate that photolysis of Diazo-2 not only lowers the free Ca^{2+} rapidly, but also suggests that in intact striated adductor muscle the rate of Ca^{2+} uptake from the sarcoplasm is the main factor limiting the rate of relaxation.

Studies of single frog skeletal muscle fibres have investigated the speed of contraction and relaxation in both intact fibres and in skinned fibres. In single fibres, the half times of relaxation were found to be 66±5 ms in intact fibres (4) and 74±6 ms using Diazo-2 in skinned fibres (10), essentially the same in both preparations. This would imply that, in contrast to the molluscan muscle, in the intact frog skeletal muscle the rate of decline of tension is not limited by the speed of the free Ca^{2+} transient.

The difference in the rates of relaxation following photolysis of 2 mM Diazo-2 between the skinned molluscan and skinned vertebrate skeletal muscle indicates that the mechanisms of relaxation may not be the same in the two muscle types.

It has been suggested that molluscan muscle may relax by the detachment of crossbridges by a reversal of the normal crossbridge cycle (14,15). However, it is not yet certain which biochemical state corresponds to the force generating state. Experiments by Sleep and Hutton (11) have indicated that all the crossbridge steps between ATP binding and P_i release are reversible (Eq.1). There is also evidence to suggest that in the absence of Ca^{2+}, scallop heavy meromyosin (HMM) can bind to scallop actin, however the ATPase activity is low (12,13) suggesting a Ca^{2+} action on a kinetic step in the ATPase cycle rather than on actomyosin binding. In addition, experiments on HMM alone have indicated that both P_i and ADP release are Ca^{2+} sensitive (Eq.1) and may be the key Ca^{2+} sensitive steps (14). Phosphate release from myosin was 0.0021 s^{-1} and ADP displacement by actin was 0.023 s^{-1} in the absence of Ca^{2+}, whilst the overall steady-state rate in the presence of Ca^{2+} was 0.3 s^{-1} (14). It seems possible that when the Ca^{2+} falls the P_i and ADP release steps are inhibited and relaxation occurs predominantly via back reactions. Vertebrate muscle crossbridges, in contrast, probably detach by completing the crossbridge cycle and deactivation of the thin filament. Thus the difference in the rates of relaxation may be due to the differing crossbridge kinetics of the relaxation process in these muscles.

If this hypothesis is correct, the process of relaxation in the scallop adductor muscle is likely to be affected by the rate at



(1)

which the crossbridge heads can detach via the proposed back reactions. The results of this experiment indicate that this process must be fast (>100 s^{-1} at 12°C) and thus not rate limiting in intact fibres. *In vivo* the Ca^{2+} transient is the rate-limiting factor for relaxation (<10 s^{-1} at 10°C).

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