Regulation of cross-bridge detachment by Ca ions at high ionic strength in molluscan catch muscle

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Received 6 November 1989; accepted 6 February 1990

Summary. Changes in the profile of equatorial intensities of X-ray diffraction from an intact, anterior byssal retractor muscle (ABRM) of *Mytilus* were examined at rest, during contracture brought about by acetylcholine (ACh) and a subsequent rigor-like contraction caused by raising the tonicity of the external solution, and after returning the tonicity to normal. The results suggest that the cross-bridges formed between thick and thin actin filaments during the ACh-contracture were maintained in the hypertonic solution and broken on decreasing the tonicity before the recovery of spacing of the actin filament lattice. A similar rigor-like contraction was induced in glycerinated ABRM by increasing salt concentration during active contraction. The rigor-like force declined rapidly when Ca^{++} concentration decreased. The results suggest that the detachment of the cross-bridge from the actin filament is regulated by Ca^{++} at high ionic strength in the ABRM.
Key words. Contraction; smooth muscle, *Mytilus edulis*.

The ABRM of *Mytilus edulis* is known to exhibit the so-called 'catch state', in which force is maintained for a long period with little energy expenditure[1,2]. The catch state resembles rigor. A previous report[3] showed that the application of a strong hypertonic solution to the ABRM during ACh or high-potassium contracture inhibited relaxation, by making the actively contracting muscle like one in a state of rigor. This effect was reversible. In this report, the effect of the hypertonic solution was studied in more detail using the technique of X-ray diffraction, both with an intact ABRM and with a glycerinated ABRM.

Materials and methods

An intact preparation was made from the ABRM of *Mytilus edulis* as described previously[3]. For X-ray experiments, the muscle was mounted vertically in an experimental chamber with two Mylar windows. A low angle camera was used with an X-ray generator (1 KW, Rigaku FR-1000) operated with a fine focus on a copper target. The equatorial X-ray diffraction pattern (specimen to detector distance, 36 cm) with an exposure time of 200–400 s, was recorded with a linear position-sensitive proportional counter[4,5]. The data were registered on a conventional multichannel analyzer and recorded on an ink-writing oscillograph. The standard solution (artificial seawater) was composed of 450 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$ at pH 7.0–7.2 adjusted by $NaHCO_3$. The muscle was made to contract by applying 10^{-4} M ACh dissolved in the standard solution. A hypertonic solution was made by adding NaCl to the standard solution. Solutions were changed by draining the chamber and refilling.

The glycerinated ABRM was made by immersing it in 80–100% glycerol after relaxing it in the standard solution containing 10^{-6} M serotonin. It was tied on to a glass rod and kept in a freezer until use. One end of the preparation, without shell and byssus, was glued with fast-drying adhesive to a force transducer (Akers AE801), and the other end was glued to a rigid glass support. The changes in force were recorded on an ink-writing oscillograph.

The relaxing solution was composed of 100 mM KCl, 1 mM $MgCl_2$, 4 mM Na_2ATP, 5 mM EGTA and 20 mM imidazole buffer at pH 7.0. An activating solution was made by adding 2.0 mM $CaCl_2$ to the relaxing solution and had pCa 6.3 (computed using the apparent stability constant (K′) for Ca-EGTA complex of $10^{6.1}$ at pH 7.0[6]. Solutions with high salt concentration were made by adding 200 or 400 mM KCl to the relaxing and to the activating solutions. K′ decreases with the increase of ionic strength, and appears to reach a fixed value symptotically[7]. If $K' = 10^{5.7}$ is assumed as the final value reached at the high ionic strengths used in this study, then pCa = 5.9.

Changes of solution were made by transferring the preparation from one trough (10 × 25 × 10 mm) to another, containing a new solution. In the following description, the solutions will be called 100 K relaxing, 100 K activating, 500 K relaxing solution and so on, according to the content of KCl. All experiments were made at room temperature (22–25 °C).

Results and discussion

The intensity profile of an equatorial diffraction pattern from a relaxed muscle showed a peak slightly smaller than $1/d = 7 \times 10^{-3}$ Å^{-1} (fig. 1 A), where d is the spacing between the actin filaments in the lattice region adjacent to the dense body[8]. On the application of ACh the intensity inside the peak increased, with a slight decrease of the peak intensity (fig. 1 B). This pattern resembles that of the rigor state of the ABRM[8]. Therefore, it is thought that myosin heads, i.e. the cross-bridges, had come too close to the actin filaments outside the lattice region during the ACh-contracture, and become attached to them. The application of a strong hypertonic solution, the osmolarity of which was 4.2 times that of the standard solution (4.2 T solution), to the ABRM during the ACh-

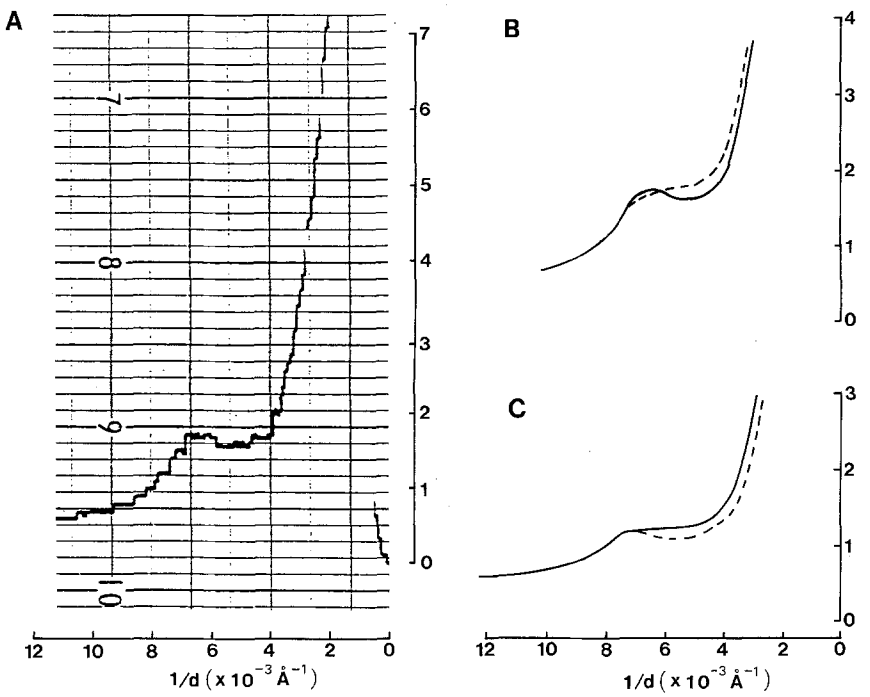

Figure 1. Equatorial intensities of X-ray diffraction from an intact ABRM. *A*, original pattern showing left half from the center of the transmitted beam. Relaxed state. *B* and *C*, smoothed tracings from oscillographs. *B*, solid curve, relaxed state traced from *A*; broken curve, early phase of ACh contracture. *C*, solid curve, a few min after application of 4.2 T solution to muscle contracted by ACh; broken curve, a few min after change from 4.2 T to the standard solution. Solid and broken curves merge on the left in both *B* and *C*. Ordinates are arbitrary units; the same in *A–C*. Abscissa, inverse of spacing.

contracture shifted the intensity profile outwards with an overall decrease of the intensities (fig. 1 C), indicating the close packing of the actin filament lattice. Returning the muscle to the standard solution resulted in a clear peak at $1/d = 7.5 \times 10^{-3}$ Å^{-1} by decreasing the intensities inside this peak (fig. 1 C).

A previous report [3] has shown that the application of a 4.2 T solution to a contracting ABRM makes it enter a state resembling rigor, and that the ABRM relaxes rapidly when the osmolarity returns to normal. Therefore, it is reasonable to conclude that the rigor-like force was maintained in the hypertonic solution by the same cross-bridges as those formed during the preceding ACh-contracture. The cross-bridges seemed to detach from the actin filaments before recovery of the spacing of the actin filament lattice, when the osmolarity was returned to normal.

In vertebrate skeletal muscle fibers, the inhibitory effect of hypertonic solutions on the mechanical activity, for example on shortening velocity, is mainly ascribed to osmotic compression of the fiber, which mechanically interferes with the interaction between the cross-bridges and the actin filaments [9, 10]. In the ABRM, the detachment of the cross-bridges appeared to be inhibited in the hypertonic solution predominantly by increased intracellular ionic strength rather than by the osmotic compression of the fiber. This agrees with the previous report [3] that the rate of relaxation in hypertonic solutions made of sucrose or NaCl is independent of the degree of shrinkage of the ABRM.

When a 100 K activating solution was applied, the glycerinated ABRM usually developed a force of about 1 kg/cm^2. An application of a 100 K relaxing solution made the muscle relax (fig. 2 B). A 300 K activating solution produced a force that was less than half of the maximum force attained in the 100 K activating solution, while a 500 K activating solution failed to produce any force development, indicating that in this case there was no attachment of the cross-bridges to the actin filaments. Such a response of the glycerinated ABRM to increasing ionic strength is qualitatively similar to that of skinned frog skeletal muscle fibers [11]. When the solution was changed from the 100 K to the 500 K activating solution during an active contraction, as shown in figure 2 C–D, a small drop in force was followed by a very slow relaxation. The force was maintained almost passively in the 500 K activating solution, as shown by a quick release method [12] (fig. 2 D). The detachment of the cross-bridge is thought to be very slow in the 500 K activating solution.

A change from the 500 K activating to a 500 K relaxing solution resulted in a rapid relaxation (fig. 2 D). The effect of increasing salt concentration on the glycerinated ABRM was quite similar to that of the hypertonic solution on the intact ABRM [3]. Such a maintenance of force was not observed when the 500 K activating solution was

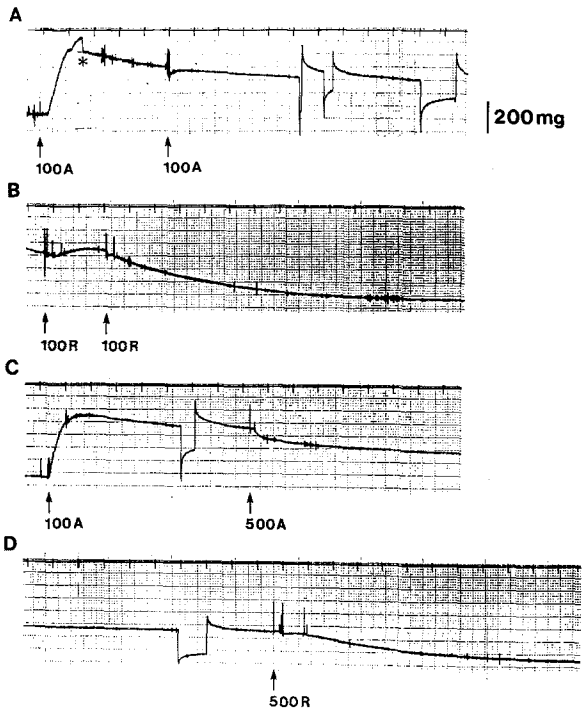

Figure 2. Effects of 500 K activating and relaxing solutions on a contracting, glycerinated ABRM. Records *A–D* are continuous. 100 K activating (100 A), 100 K relaxing (100 R), 500 K activating (500 A) and 500 K relaxing (500 R) solutions were applied at arrows. Position of the pen was changed slightly at the asterisk in *A*. A quick length reduction and restretch were applied manually in *A*, *C* and *D* as recognized by quick force changes. Time marks, 1-min intervals.

applied to a contracting, glycerinated rabbit psoas muscle fiber. Such a difference in the response to the increasing ionic strength between the glycerinated ABRM and rabbit fibers may be attributed to the difference in the function of the myosin head (cross-bridge); the Ca regulation site resides in the myosin head in the former[13], but in the thin filament in the latter[14].

In the ABRM, it would be expected that the interval between the detachment of Ca^{++} from and the binding of new Ca^{++} to the cross-bridge would be prolonged if the Ca^{++} concentration in the surrounding solution decreases. Therefore the present result that, in the solution with a high salt concentration, the cross-bridges detach very slowly in the presence of Ca^{++}, and rapidly upon the withdrawal of Ca^{++}, implies that the Ca bound to the cross-bridge inhibits either ADP release from it, or ATP binding to it, suppressing the subsequent detachment of the cross-bridge from the actin filament. Such an interaction between the bound Ca and the nucleotides may not only occur with ABRM in a high-concentration salt solution, but be applicable to the muscle under physiological conditions, since the rate of relaxation and therefore the rate of cross-bridge detachment decreases with an increase of the tonicity of the bathing solution in a graded manner in the intact ABRM[3]. Structural changes occur in the myosin heads when they bind Ca^{++}[15]. The binding of Ca could alter the structure in the ATP binding region in the myosin head, preventing either ADP release from the myosin head, or the binding of ATP to it.

Acknowledgments. I would like to thank Prof. H. Sugi for facilities and Drs H. Tanaka and H. Hashizume for their kind help with the X-ray experiments.

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0014-4754/90/070677-03$1.50 + 0.20/0