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EFFECT OF CALCIUM IONS ON RIGOR CONTRACTIONS IN SKINNED FROG MUSCLE FIBERS

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Summary

Rigor contractions were examined in skinned frog muscle fibers. The concentrations of calcium ions, pCa = 9.0—5.0, in the solutions which caused rigor were shown to affect the magnitude and time course of the contractions.

Rigor is the state in muscle in which myosin cross bridges are attached to the thin filaments of actin in the absence of ATP. White [1] examined what was termed 'rigor contractions' in glycerinated muscle fibers of the water bug, *Lethocerus*; when a muscle was moved from a relaxing solution containing ATP to a solution with no ATP, the muscle contracted as it went into rigor. Bremel and Weber [2] showed that the formation of rigor linkages placed thin filaments in a potentiated state in which ATP hydrolysis occurred even in the absence of calcium ions, while troponin was shown to bind calcium ions with increased affinity in the presence of rigor linkages. This paper reports that the magnitude and time course of rigor contractions in skinned frog muscle fibers vary as a function of the concentration of calcium ions in the bathing medium.

Single muscle fibers of the frog were isolated and skinned as described by Endo, Tanaka and Ogawa [3] and Julian [4]. The relaxing solution was composed of potassium methanesulfonate, 100 mM; imidazole, 20 mM; $MgCl_2$, 4 mM; Na_2ATP, 4 mM and K_2EGTA, 2 mM; it had a pH of 7.0 and a pCa = 9.0 (the binding constant of EGTA for calcium was $5 \cdot 10^6$ M^{-1}; contaminant calcium was assumed to be 10^{-5} M). The rigor solutions had no ATP; they were composed of potassium methanesulfonate, 106 mM; imidazole, 20 mM; $MgCl_2$, 4 mM and K_2EGTA and $K_2CaEGTA$ in appropriate concentrations so that the total concentration of EGTA was 2 mM and the pCa was 9.0, 8.0, 7.7, 7.0, 6.0 or 5.0. The pH of the rigor solutions was 7.0, and the temperature of all solutions was 22°C. Segments of mechanically and chemically skinned fibers were mounted in an apparatus that allowed the segments to be moved rapidly between different bathing solutions. The tension produced by the segments was measured with a force transducer as each segment was bathed in the different solutions.

Fig. 1 illustrates the results of an experiment in which a chemically skinned segment was bathed alternately in the relaxing solution and rigor solutions which had different concentrations of calcium. Fig. 2 summarizes the results of this and three similar experiments. A contraction occurred each time the segments were exposed to a rigor solution. The magnitude of the tension and the rate at which it developed were directly related to the concentrations of calcium ions in the rigor solutions. While they were in the rigor solutions and as the tension increased, the segments became white and their stiffness increased. The tension produced by a segment was approximately the same each time the segment was bathed in a particular rigor solution. All segments relaxed each time they were returned to the relaxing solution.

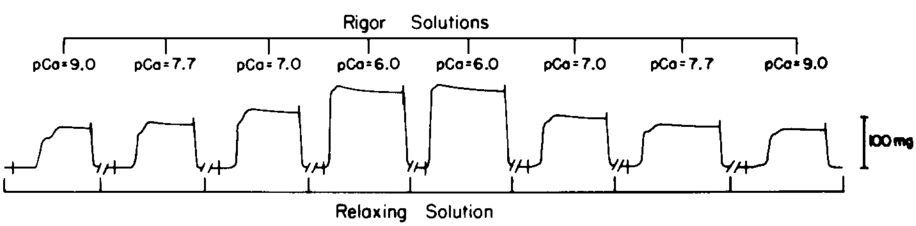

Fig. 1. Results of an experiment on a chemically skinned segment. The segment was bathed in rigor solutions with different calcium concentrations, and a contracture occurred in each solution. The segment remained in each solution for 5 min; it relaxed in the relaxing solution. The short vertical lines in the record indicate when the solutions were changed.

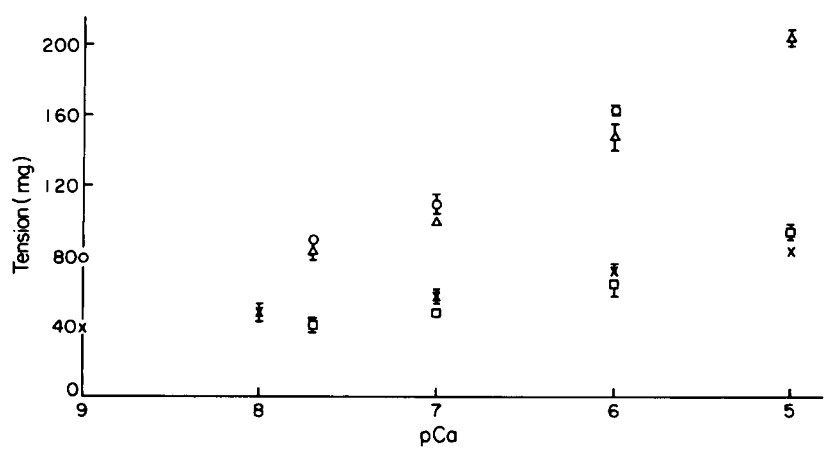

Fig. 2. Summary of the results of experiments from 2 chemically skinned (○, △) and two mechanically skinned (□, X) segments of 3 skinned fibers. The segment of Fig. 1 is represented by ○. Each point represents the mean of the maximum tensions produced by the segment each of the two times the segment was in that solution. The exception regarding the usual pattern of the delay in rigor solution prior to contraction (see text) occurred in the segment symbolized by X: the second time the segment was placed in the rigor solution with a pCa = 9.0, the delay was shorter than the delay in the previous rigor solution with a pCa = 8.0.

With one exception (see legend of Fig. 2), the delay between the time a segment entered a rigor solution and the time tension began to increase rapidly was inversely related to the calcium concentration in the rigor solution.

These results showed that rigor contractions occurred in skinned frog muscle fibers at a very low calcium concentration, pCa = 9.0; however, they also showed that calcium ions at low concentrations, pCa = 9.0—7.0, as well as the concentrations, pCa = 7.0—5.0, influenced the magnitude and time course of the rigor contractions. When the pCa was between 9.0 and 7.0, the control of tension by calcium was not as great as the control between 7.0 and 5.0; for this reason, each segment was exposed not only to increasing concentrations of calcium in rigor solutions, but to decreasing concentrations as well. The results eliminated the possibility that the magnitude of tension produced in one rigor solution depended on the calcium concentrations of the solutions to which the segments had previously been exposed.

These results on rigor contractions in skinned fibers are consistent with the results of Bremel and Weber [2]. Rigor linkages potentiated the thin filaments for production of tension; the ATP which was transferred with the segments when they were moved from relaxing solution to rigor solutions provided the energy for the contracture [1]. Since the binding constant for one of the calcium binding sites on troponin increases in the presence of rigor linkages, tension was controlled by calcium ions at a lower concentration than when the ATP concentration was normal.

No further increase in tension was observed when the pCa was below 5.0. One reason for the apparent control by calcium at the higher concentrations was possibly that the magnitudes of pCa, as calculated, indicated only the maximum possible calcium concentrations in the segments; the true calcium concentrations within the segments were not known. Alternatively, it is possible that part of the tension that was generated in the rigor solution with pCa = 6.0 and 5.0 was that of an ordinary contraction of the skinned segments. When the segments were moved from the relaxing solution to the rigor solutions the segments themselves presumably had an ATP concentration of 4 mM. Although the ATP concentration was quickly diluted in the rigor solutions, it may have remained sufficiently high for a sufficient length of time that an ordinary contraction could have occurred in the presence of the high calcium concentrations in the rigor solutions with pCa = 6.0 and 5.0.

These experiments were similar to those of Godt [5] who showed that at low concentrations of ATP, the control of tension was extended to low concentrations of calcium. However, calcium control was not evident after the production of rigor contractions was complete. Presumably the lack of control occurred because calcium had no effect on established rigor linkages. These experiments show that calcium influences the rigor contractions themselves.

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