Cooperativity of the calcium switch of regulated rabbit actomyosin system

J. M. Murray and A. Weber
Medical Research Council, Cambridge, England and Department of Biochemistry and Biophysics, University of Pennsylvania

Summary

The concentration range required for calcium activation of skeletal myofibrillar ATPase activity has previously been attributed to simultaneous binding of two calcium ions to each troponin. We present data representative of the majority of myofibrillar preparations and data with acto subfragment-1 (S-1) whose calcium activation of ATPase activity occurs over a much too narrow range of calcium concentrations to be so explained. S-1 binding significantly broadened the range of Ca^{2+} concentrations over which activation occurred but not to the extent that is associated with simultaneous binding of 2 calcium ions.

Introduction

ATPase activity of rabbit myofibrils rises as a steep function of calcium concentration (1). Since there is no positive cooperativity of calcium binding either to pure troponin (2) or to the actin bound troponin-tropomyosin complex (3, 4) the sharpness of the transition has been attributed to the fact that 2 (2) or 4 calcium ions (5) must be simultaneously bound for activation. In the discussion to follow it is helpful to think of the troponin-tropomyosin complex as a 'switch' which turns the actin filament on and off in response to changes in calcium concentration. The steeper the slope of activity plotted versus calcium concentration, the more nearly does this regulatory system approach the behavior of a simple binary device, an on-off switch.

We will first present calcium responses of the switch that are much too steep to be explained by a requirement for simultaneous binding of even 4 calcium ions. Secondly, we will show how myosin saturation of the actin filament affects the steepness of the switch.

Materials and methods

The proteins and the reconstituted regulated actin filaments were prepared as previously described (4,7). ATPase activity was measured in the presence of 5 mM creatine phosphate, 1 mg/ml creatine kinase, 10 mM imidazole pH 7.0, 25°. Myofibrils contained 5 mM Mg^{2+} in excess over MgATP and the ionic strength was adjusted to 90 mM with KCl. Acto-S-1 contained 1 mM Mg^{2+} in excess over MgATP and the ionic strength was adjusted to 30 mM.

Results and discussion

Figure 1 shows the calcium activation of myofibrillar ATPase activity at different ATP concentrations. It is immediately obvious that for all but two curves ATPase activity increased as a rather steep function of calcium concentration: at 2 mM MgATP ATPase activity increased from 10 to 90 per cent of maximal over an 8 fold range of calcium concentrations instead of the 30–35 fold range that

Molecular and Cellular Biochemistry 35, 11–15 (1980). 0300-8177/81/0351-0011/$01.00.

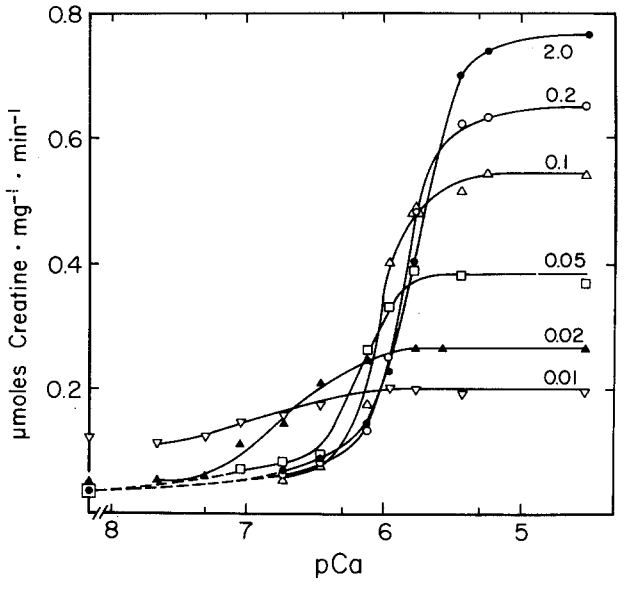

Fig. 1. Calcium titration of myofibrillar ATPase activity with decreasing ATP concentrations. Free calcium ion concentrations were calculated from the ratios EGTA/CaEGTA using the constants given in Chaberek and Martell (9). Numbers on curves indicate mM ATP concentrations.

would be obtained if the switching on of ATPase activity required only the simultaneous binding of 2 or 4 calcium ions for each troponin molecule.

Furthermore, the response to Ca^{2+} became steeper with increasing activation, i.e. to raise activity from 50 to 90 percent required only a 2 fold increase in calcium concentration as compared to a 4 fold increase for activation from 10 to 50 per cent (Table 1). By contrast, a requirement for the simultaneous binding of several calcium ions affects the response to calcium in the opposite manner: it is steepest between 10 and 50% activation and becomes broader at higher degrees of activation (Table 1).

How does one explain the much shallower calcium response previously reported (2), which is accounted for by a requirement for simultaneous binding of 2 calcium ions? The steepness of the response apparently is subject to alterations (10): the earliest calcium titration of ATPase activity of reconstituted actomyosin can be described by calcium binding to a single site (11). The reason for this variability has not yet been found. We are inter-

Table 1. Observed increments of calcium concentrations necessary to cause designated increases in ATPase activity, compared to calculated increments assuming a requirement for simultaneous binding of either 2 or 4 calcium ions.

		Increases in Ca^{2+} concentration (in multiples) required to produce designated increases in ATPase activity (% V_{max})						Percent relaxation[a]
Actomyosin	mM MgATP	10–90%		10–50%		50–90%		
		Observed						
myofibrils	0.02	12		3		4		82
	0.05	12		5		2.5		90
	2.0	7		3.5		2.1		95
10 μM regulated actin + 0.2 μM S-1	1.0	7.7		3.5		2.2		95
2 μM regulated actin + 100 μM S-1	0.5	14		5.3		2.5		43[b]
	2.0	13.3		4		3.5		79
		Calculated for simultaneous binding of						
		2	4	2	4	2	4	calcium ions
		35	30	5.1	4	7.5	7.5	

[a] % relaxation = 100 (1 - EGTA/Ca^{2+}); [b] the decrease in relaxation is due to the combination of a high S-1/actin ratio and a low ATP concentration, i.e. an increased concentration of acto-S-1 (rigor complexes).

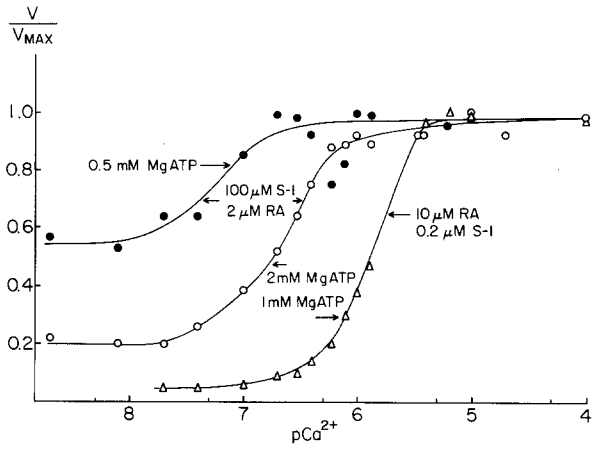

Fig. 2. Calcium titration of acto-S-1 ATPase activity. V_{max} indicates the rate at calcium saturation for each set of conditions.

ested in the explanation for the steepest responses that have been observed.

The steepness of the calcium response does not depend on the structural organization as can be seen from the calcium titration curve of the ATPase activity of regulated acto-S-1 (Fig. 2). When actin was in excess over S-1, ATPase activity increased from 10 to 90 per cent over an 8 fold range of calcium concentrations. Nor did solubilization alter the increasing steepness of the calcium response with increasing activation (Table 1). This distinguishes the actin activated system from the myosin regulated system of the scallop where Chantler and his colleagues (12) observed a considerable flattening of the switch when it was measured with HMM instead of thick filaments.

The second prominent feature of Fig. 1 is a shift in the midpoint of the calcium titration curves to lower calcium concentrations with decreasing ATP saturation (The latter is indicated by decreasing rates of ATP hydrolysis after saturation with calcium). When ATP is no longer saturating the lifetimes and, therefore, the steady state concentrations of nucleotide-free acto-S-1 complexes (rigor complexes) increase (9). If the actin filaments contain tropomyosin or troponin and tropomyosin, such complexes, by cooperative action, markedly modify actin activation of myosin ATPase activity and calcium binding (4, 7). First, the rigor complexes cause potentiation of ATPase activity (7, 15, 16) and an increased affinity of nucleotide-free S-1 for tropomyosin-actin (15) as well as of tropomyosin for actin (17) and second, when the rigor complexes accumulate on the tropomyosin-actin filament they abolish relaxation (4, 15) in the absence of calcium, at the same time increasing the calcium binding constant of troponin (4).

The first effect must involve conformational changes in either actin or tropomyosin (15) whereas reversal of relaxation in the absence of calcium, i.e. turning on of the troponin-tropomyosin switch by rigor complexes rather than by calcium ions can be visualized without invoking conformational changes in tropomyosin or actin. As discussed elsewhere (18) it is possible that in the absence of Ca^{2+} rigor complexes compete successfully with tropomyosin-troponin for sites on the periphery of the actin filament, thus displacing tropomyosin-troponin into the groove. At saturating ATP concentrations when the concentration of rigor complexes is mimimal, that displacement of tropomyosin into the groove can only be achieved by calcium saturation of troponin C which sets the following chain of events into motion: a very rapid conformational change in troponin C (19) inducing a conformational change in troponin I (20) which results in the dissociation of troponin I from its actin binding site (21) allowing the displacement of tropomyosin into the groove (22, 23, 24).

The energy necessary for the conformational change of troponin is supplied by calcium binding, i.e. the binding constant of calcium for troponin must be reduced by a value that corresponds to the energy necessary for the conformational transitions of the troponin molecule. When these conformational changes have occurred as the result of the displacement of the troponin-tropomyosin complex by S-1 or myosin (20) all of the binding energy of subsequently bound Ca^{2+} must be released thus increasing the binding constant of calcium for troponin.

When rigor complexes have completely switched on the troponin-tropomyosin switch one can, of course, no longer measure the response of the switch to Ca^{2+}. It is of interest, however, to know whether the calcium response is altered when the actin filaments have been partially switched on by rigor complexes (as indicated by a reduction in the per cent of relaxation, Table 1, Fig. 2) or when only the midpoint of the calcium titration curve has been shifted to lower calcium concentrations without

significant reversal of relaxation (Fig. 1). The experiments of Fig. 2 were designed to study both variables. When we increased the ratio of S-1 to actin from 0.02 to 50 the Ca^{2+} concentration for 1/2 activation was reduced 5 fold, associated with a small reduction in relaxation. If, in addition, the concentration of ATP was reduced to 0.5 mM 50% of the actin molecules were switched on and the midpoint of the titration curve was lowered to 1/20 of that observed with a low S-1/actin ratio. Shifting of the midpoint of titration to lower calcium concentrations significantly broadened the range of calcium concentrations for 10–90% ATPase activation from 7–8 fold to 12–14 fold. Nevertheless, the range remained much narrower than the 35 fold range associated with activation by the simultaneous binding of 2 calcium ions. Broadening seemed to be maximal with a relatively small shift in the midpoint of the titration curve (Table 1, Fig. 1) and did not increase in parallel with further shifts to lower calcium concentrations. Furthermore, there did not seem to be an additional effect on the steepness of the response by partial activation of the troponin-tropomyosin-actin filaments by rigor complexes in the absence of Ca^{2+}. (The only exception, one curve in Fig. 1, does not have enough points to be taken into account.)

In conclusion, these data show clearly that it is impossible to explain the calcium response of the troponin-tropomyosin switch solely by calcium binding to troponin. It is quite likely, as suggested first by Tawada and his colleagues (14), that the calcium response is modified by tropomyosin-tropomyosin interactions since all tropomyosin molecules are interconnected into one long strand (for ref. see 22). Tawada and his colleagues (14) determined the effect of removing the overlapping ends of tropomyosin on the calcium activation of superprecipitation. Although they found some broadening with shortened tropomyosin the calcium response remained still very steep. This, however, may be a consequence of the method of measurement, i.e. turbidity changes which are more complex than measurements of ATPase activity.

Recently, binding curves obtained with S-1-ADP and regulated actin in the absence of calcium have been modelled using tropomyosin-tropomyosin interactions (25).

The physical basis for such cooperative tropomyosin-tropomyosin interactions may be the relative (but not absolute, (26)) stiffness of the tropomyosin molecule, a coiled coil, which has been invoked to explain other cooperative effects involving tropomyosin (16). A single Ca^{2+}-saturated troponin-tropomyosin complex that is surrounded by two Ca^{2+}-free troponins, firmly bound to actin, can move into the groove and vacate all seven associated myosin bindings sites only if the tropomyosin strand forms relatively sharp bends. If tropomyosin is not floppy enough to do that but can form only shallow bends, only some of the associated myosin binding sites will be completely uncovered under these conditions (Fig. 3A). When, on increasing calcium saturation, neighboring troponin molecules bind Ca^{2+} and become dissociated from actin large stretches of myosin binding sites become uncovered, greatly increasing the number of vacated sites per troponin molecule (Fig. 3B).

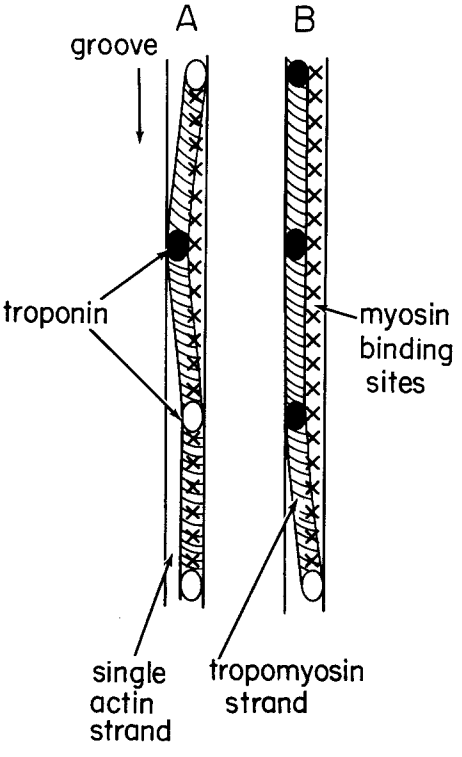

Fig. 3. Single troponin-tropomyosin actin strand with the groove at the left of each strand. Filled in ellipsoid = Ca^{2+} saturated troponin molecules, open ellipsoids = Ca^{2+}-free troponin. *A*, only 1–3 myosin binding sites (crosses) completely vacated by 1 Ca^{2+} saturated troponin-tropomyosin complex; *B*, about 15 myosin binding sites vacated by 3 neighboring Ca^{2+} saturated troponin-tropomyosin complxes.

Within this framework it is possible to also explain the effects of isolated rigor complexes on the calcium response. However, such detailed discussion will be postponed until experiments proving or disproving such tropomyosin-tropomyosin interactions have been performed.

Acknowledgements

This work was supported by NIH grant HL 15692.

A preliminary report of this work was presented at an International Symposium sponsored by the University of Wisconsin, 1969 and at the International Symposium on Calcium Transport in Contraction and Secretion at Bressanone, Italy, 1975.

References

1. Weber, A. and Herz, R., 1963. J. Biol. Chem. 238: 599–605.
2. Potter, J. D. and Gergely, J., 1975. J. Biol. Chem. 250: 4628–4633.
3. Weber, A. and Bremel, R. D., 1971. In: Contractility of Muscle Cells and Related Processes (R. J. Podolsky, ed.) pp. 37–53.
4. Bremel, R. D. and Weber, A., 1972. Nature (New Biol.) 238: 97–101.
5. Weber, A. and Murray, J. M., 1973. Physiol. Rev. 53: 612–673.
6. Weber, A., 1970. In: The Physiology and Biochemistry of Muscle as a Food, 2 E. J. Briskey, R. G. Cassens and B. B. Marsh, eds.) Univ. Wisconsin press pp. 383–394.
7. Bremel, R. D., Murray, J. M. and Weber, A., 1972. Cold Spring Harbor Symp. Quant. Biol. 37: 267–275.
8. Chaberek, S. and Martell, A. E., 1959. In: Organic Sequestering Agents. John Wiley, NY.
9. Taylor, E. W., 1979. Crit. Rev. Biochem. 6: 103–164.
10. Weber, A., Herz, R. and Reiss, I., 1964. Fed. Proc. 23: 896–900.
11. Weber, A. and Winicur, S., 1961. J. Biol. Chem. 236: 3198–3202.
12. Chantler, P. D., Sellers, G. R., Szent Gyorgyi, A. G., in press.
13. Pemrick, S. and Weber, A., 1976. Biochemistry 15: 5193–5198.
14. Tawada, Y., Ohara, H., Oai, T. and Tawada, K., 1975. J. Biochem. 78: 65–72.
15. Murray, J. M., Weber, A. and Wegner, A., in press.
16. Murray, J. M., Knox, M. K., Trueblood, C. and Weber, A., 1980. FEBS Lett. 114: 169–173.
17. Eaton, B. L., 1976, Science 192: 1337–1339.
18. Murray, J. M., Knox, M. K. and Weber, A., in press.
19. Johnson, J. D., Charlton S. C. and Potter, J. D., 1979. J. Biol. Chem. 254: 3497–3502.
20. Trybus, K. M. and Taylor, E. W., 1980. Fed. Proc. 39: 1816.
21. Potter, J. D. and Gergely, J., 1974. Biochemistry 13: 2697–2703.
22. Parry, D. A. D. and Squire, J. M., 1973. J. Mol. Biol. 75: 35–55.
23. Huxley, H. E., 1972, Cold Spring Harbor Symp. Quant. Biol. 37: 361–376.
24. Haselgrove, J. C., 1972. Cold Spring Harbor Symp. Quant. Biol. 37: 341–352.
25. Hill, T. L., Eisenberg, E. and Greene, L. E., 1980. Proc. Natl. Acad. Sci. 77: 3186–3190.
26. Phillips, G. N., Jr., Lattman, E. E., Cummins P., Lee, K. Y. and Cohen, C., 1979. Nature 278: 413.

Received August 11, 1980.