THE TIME-COURSE OF Ca^{2+} EXCHANGE WITH CALMODULIN, TROPONIN, PARVALBUMIN, AND MYOSIN IN RESPONSE TO TRANSIENT INCREASES IN Ca^{2+}

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ABSTRACT We have modeled the time-course of Ca^{2+} binding to calmodulin, troponin, parvalbumin, and myosin in response to trains of transient increases in the free myoplasmic calcium ion concentration (pCa). A simple mathematical expression was used to describe each pCa transient, the shape and duration of which is qualitatively similar to those thought to occur in vivo. These calculations assumed that all the individual metal binding sites are noninteracting and that Ca^{2+} and Mg^{2+} bind competitively to the Ca^{2+}-Mg^{2+} sites of troponin, parvalbumin, and myosin. All the on-and-off rate constants for both Ca^{2+} and Mg^{2+} were obtained either from the literature or from our own research. The percent saturation of the Ca^{2+}-Mg^{2+} sites with Ca^{2+} was found to change very little in response to each pCa transient in the presence of 2.5×10^{-3}M Mg^{2+}. Our analysis suggests that the Ca^{2+} content of these sites is a measure of the intensity and frequency of recent muscle activity because large changes in the Ca^{2+} occupancy of these sites can occur with repeated stimulation. In contrast, large rapid changes in the amount of Ca^{2+} bound to the Ca^{2+}-specific sites of troponin and calmodulin are induced by each pCa transient. Thus, only sites of the "Ca^{2+}-specific" type can act as rapid Ca^{2+}-regulatory sites in muscle. Fluctuation in the total amount of Ca^{2+} bound to these sites in response to various types of pCa transients further suggests that in vivo only about one-half to one-third of the total steady-state myofibrillar Ca^{2+}-binding capacity exchanges Ca^{2+} during any single transient.

INTRODUCTION

It is well established that the free myoplasmic calcium ion concentration (pCa) of the milieu surrounding the myofilaments regulates contractile activity of muscle. A large body of literature exists describing the relationship between steady-state levels of pCa and various correlates of contraction such as myofibrillar ATPase (1, 2) and force development in segments of skinned (i.e., with sarcolemma removed) muscle cells (1, 3). As steady-state levels of pCa decrease from ~8 to ~5 these functions increase from basal to maximal activity over the same pCa range that cardiac (2) and skeletal (4) myofibrils bind significant amounts of Ca^{2+}.

Coincident with Ca^{2+} activation of actomyosin ATPase, Ca^{2+} binds to several different classes of myofibrillar Ca^{2+}-binding sites. It is therefore important to know the physiological role each class may play in regulating Ca^{2+}-activated force. Because myoplasmic pCa fluctuates rapidly in active muscle (5–9), constraints on the physiological functions of these

Ca^{2+}-binding sites can be further characterized by determining the time course of Ca^{2+} exchange with each class of sites in response to pCa transients similar to those thought to occur in active muscle.

The purpose of this computer analysis was to follow the time-course of the percentage of maximal occupancy of each class of sites with Ca^{2+} in response to various pCa transients whose shape and duration were qualitatively similar to those which occur in active muscle. The physiological functions ascribed to the various class of sites based upon steady-state Ca^{2+}-binding data (2, 10–13) are, in some cases, quite different from those suggested by our kinetic modeling analysis.

Almost all the Ca^{2+} bound to the myofibrils in the presence of $\sim10^{-5}$M Ca^{2+} is associated with troponin (14), although some (depending upon $[Mg^{2+}]$) may be bound to myosin (11, 12). The Ca^{2+}-binding sites on both skeletal and cardiac troponin separate into two classes based upon the extent to which physiological levels of Mg^{2+} (i.e., $\sim10^{-3}$M) reduce their apparent affinity for Ca^{2+}. These proteins each contain two high affinity Ca^{2+}-Mg^{2+} sites whose apparent affinity for Ca^{2+} is reduced by millimolar Mg^{2+} (10, 15). They also contain Ca^{2+}-binding sites that are not influenced by this level of Mg^{2+}. Skeletal troponin contains two (10), whereas the cardiac protein has only one of the Ca^{2+}-specific class of sites (15, 16). Myosin from both tissues binds two gram-atoms of Ca^{2+} with relatively high affinity. Even though these sites on myosin also have a high affinity for Mg^{2+}, they can contribute substantially to the myofibrillar Ca^{2+}-binding capacity when steady-state pCa levels are low enough to activate muscle fully (2, 11, 12, 17).

Two other non-myofibrillar proteins, calmodulin and parvalbumin, have been included in our analysis, because they play an indirect role in Ca^{2+} regulation of contraction. Ca^{2+} regulates a multitude of cellular activities via binding to calmodulin (18). The Ca^{2+}-binding properties of calmodulin have been the subject of several studies and although there are some inconsistencies in the number of high and low affinity sites, most studies agree that the metal-binding properties of calmodulin are similar to those of the Ca^{2+}-specific sites of troponin (19, and references cited therein), and that it contains four Ca^{2+}-specific sites per molecule. Calmodulin, however, is present in muscle at a much lower concentration than troponin (20).

Parvalbumins are soluble Ca^{2+}-binding proteins of low molecular weight found primarily in white muscles in a wide variety of animals (21). The properties of the two Ca^{2+}-binding sites of parvalbumin are similar to those of the Ca^{2+}-Mg^{2+} sites of whole troponin (19, 22, 23, and references cited therein). Although the physiological function of the parvalbumins is not yet known, they are important because when present they constitute a major Ca^{2+} sink, rivaling that of troponin (in the turtle there is ten times more parvalbumin than troponin [21]). The equilibrium divalent metal-binding properties and cellular content of these four proteins, as used in this analysis, are summarized in Table I.

Two parameters concerning the Ca^{2+} content of these different classes of Ca^{2+}-binding sites can be defined. The first and the easiest to measure is the total amount of Ca^{2+} bound as a function of steady-state pCa. This relationship has been determined separately for all the Ca^{2+}-binding sites on the individual proteins mentioned above and for intact myofibrils. The second parameter, the amount of Ca^{2+} required to activate muscle as a function of pCa, is less than the total Ca^{2+} bound because only certain sites are thought to regulate force and ATPase

TABLE I

CELLULAR CONTENT AND METAL-BINDING PROPERTIES OF FOUR PROTEINS

Protein	Cellular content, wet wt.	Number of sites	Metal	K_A	k_{on}	k_{off}
	(μM/kg)			*(M^{-1})*	*($M^{-1}\,s^{-1}$)*	*(s^{-1})*
Calmodulin	5 (18)	4	Ca	4.2×10^5 (19)	1×10^{8}*	238
Troponin						
Skeletal	70 (34)	2	Ca	5×10^6 (10)	1.15×10^8	23.0 (31)
		2	Ca	5×10^8 (10)	3.0×10^8	0.6 (31)
			Mg	5×10^4 (10)	1×10^5*	2.0
Cardiac	28 (17)	1	Ca	2×10^6 (15)	3.9×10^7	19.6‡
		2	Ca	3×10^8 (15)	1×10^8*	0.33
			Mg	3×10^3 (15)	1×10^5*	3.33
Parvalbumin	§	2	Ca	2.5×10^8 (31)	2.5×10^8	1.01 (30)
			Mg	1.1×10^4 (31)	6.6×10^4	6.0 (30)
Myosin	‖	2	Ca	3×10^7 (11)	1.38×10^7	0.46 (34)
			Mg	2.75×10^5 (11)	1.57×10^4	0.057 (34)

A list of the cellular content, metal-binding capacity of each class of sites, and the rate constants used in this analysis. The references for each value are shown in parentheses.

*k_{on} was assumed whenever measurements for k_{off} were unavailable. In all cases the relationship $K_A = k_{on}/k_{off}$ was used to calculate K_{on}.

‡Unpublished observation.

§Not included in the slow model but equal to troponin in the fast model.

‖Equal to troponin in each model (33).

in muscle (17). The amount of bound Ca^{2+} required to activate muscle fully has been estimated at 90 μmol/kg wet weight for heart and about 300 μmol/kg wet weight for skeletal muscle (17). These estimates are based upon steady-state myofibrillar Ca^{2+}-binding data obtained over the same pCa range at which Ca^{2+}-activated force and myofibrillar ATPase vary between basal and maximal activity. In other words, these values suggest that three or four Ca^{2+} ions must bind to each troponin molecule in cardiac and skeletal muscle, respectively, to achieve maximal steady-state activation. As an extension of this suggestion, the amount of Ca^{2+} required to activate fast-twitch muscle when parvalbumin is present would therefore be higher than that predicted solely by the myofibrillar Ca^{2+}-binding capacity.

These conclusions, based upon steady-state Ca^{2+}-binding data, do not accurately represent either the amount of Ca^{2+} required for activation or the total amount of Ca^{2+} bound by the myofibrils in vivo, because (*a*) pCa varies continuously during a twitch (5-9, 24), and (*b*) the rate constants for Ca^{2+} exchange with the various classes of sites differ greatly (see Table I).

DESCRIPTION OF THE COMPUTER MODEL

Although an exact mathematical description of an in vivo pCa transient for either skeletal or cardiac muscle is not known, the response of photoproteins (5–7, 24) and metalochromic dyes (8–9) indicates that during a twitch pCa falls transiently before force has returned to resting levels. For this analysis, we have approximated the pCa transient with the following equation:

$$\mathrm{pCa}(t) = \mathrm{pCa}_{\mathrm{relax}} - A \cdot (e^{-t/f} - e^{-t/r})$$

where $pCa = -\log_{10}[Ca^{2+}]$, pCa_{relax} is the steady-state resting level of pCa, assumed to be 8, A is an amplitude factor, and r and f are the rising and falling time constants, respectively. It must be emphasized that the qualitative conclusions we draw are completely independent of the shape or duration of the pCa transient. But precise quantitative assessments of in vivo Ca^{2+} fluxes associated with Ca^{2+} binding to myofibrillar proteins must be delayed until an exact description of a pCa transient is determined in the same muscle from which these proteins are obtained.

The time-course of the percent saturation of each class of sites with Ca^{2+} was calculated on the assumptions that all the individual metal binding sites are noninteracting, that Ca^{2+} and Mg^{2+} bind competitively to the Ca^{2+}-Mg^{2+} sites of troponin, parvalbumin, and myosin (10–12, 15, 19), and that $[Mg^{2+}]$ remained constant at 2.5×10^{-3} mol/liter of cell water (25). The assumption of no cooperativity in Ca^{2+} binding to each of the individual proteins is justifiable. The presence of millimolar Mg^{2+} eliminates cooperativity in Ca^{2+} binding to myosin (12), and diminishes it in the only report of cooperative Ca^{2+} binding to calmodulin (26). No cooperativity has been reported for Ca^{2+} binding to parvalbumin (19). Finally, the cooperativity present for Ca^{2+} binding to cardiac troponin C is eliminated upon formation of the troponin complex (15). We have not incorporated the effects that the actomyosin interaction may have on the Ca^{2+}-binding properties of troponin (27) and therefore present this analysis only as representing the time-course of Ca^{2+} binding to the individual proteins.

A fast pCa transient drove the model containing the skeletal rate constants for the Ca^{2+}-specific and Ca^{2+}-Mg^{2+} sites of troponin, whereas those for cardiac troponin were used with a slower pCa transient, since the cardiac transient is slower (5). The time constants r and f were chosen so that pCa (t) attained a minimum value of 5.2 (7) in the fast model or 5.7 (24) for the slow model within a few milliseconds, and returned essentially to pCa_{relax} within 50 or 800 ms for the fast and slow models, respectively. The values of A, r, and f used to generate the fast transient were 3.26, 0.5, and 15 ms, respectively, and those for the slow transient were 2.56, 3, and 170 ms, respectively.

The following set of equations were integrated on a digital computer to obtain the percent saturation of each class of sites with Ca^{2+} as a function of time:

$$\mathrm{Ca}(t) = 10^{-\mathrm{pCa}(t)},$$

$$\mathrm{Mg}(t) = 2.5 \times 10^{-3}\mathrm{M},$$

$$\mathrm{dCaX}(t)/\mathrm{d}t = K_{\mathrm{Xon}_{\mathrm{Ca}}} \cdot \mathrm{Ca}(t) \cdot [100 - \mathrm{CaX}(t)] - K_{\mathrm{Xoff}_{\mathrm{Ca}}} \cdot \mathrm{CaX}(t),$$

$$\mathrm{dCaY}(t)/\mathrm{d}t = K_{\mathrm{Yon}_{\mathrm{Ca}}} \cdot \mathrm{Ca}(t) \cdot [100 - \mathrm{CaY}(t) - \mathrm{MgY}(t)] - K_{\mathrm{Yoff}_{\mathrm{Ca}}} \cdot \mathrm{CaY}(t),$$

$$\mathrm{dMgY}(t)/\mathrm{d}t = K_{\mathrm{Yon}_{\mathrm{Mg}}} \cdot \mathrm{Mg}(t) \cdot [100 - \mathrm{CaY}(t) - \mathrm{MgY}(t)] - K_{\mathrm{Yoff}_{\mathrm{Mg}}} \cdot \mathrm{MgY}(t),$$

where X represents the Ca^{2+}-specific class of sites for either skeletal or cardiac troponin or calmodulin; Y represents the Ca^{2+}-Mg^{2+} class of sites for parvalbumin, myosin, or those of skeletal or cardiac troponin; CaX(t) and CaY(t) are the percent saturations of the X and Y classes of sites with Ca^{2+}, respectively; and MgY(t) is the percent saturation of the Y class of sites with Mg^{2+} and pCa(t), as described above. Table I contains all of the k_{on} and k_{off} rate constants used in our analysis. The initial values of CaX, CaY, and MgY were their steady-state values when $pCa = pCa_{relax}$.

The time course of the percent saturation of these Ca^{2+}-binding sites in response to a train of pCa transients, such as exist in a beating heart or in repetitively stimulated skeletal muscles that are not tetanized, was also simulated. In this case the calculations for the response to a single transient were repeated until the CaX(t), CaY(t), and MgY(t) functions all attained steady-state profiles. This was accomplished by setting the initial values of CaX, CaY, and MgY in one cycle to their final values of the previous cycle. pCa(t) was made a continuous function of time throughout the simulation by shifting the second and subsequent pCa(t) curves ahead in time (i.e., $t = t + t_0$). t_0 increased from 0 to a maximum of 19 μs in the fast model or to 38 μs in the slow model.

RESULTS AND DISCUSSION

We have estimated both the Ca^{2+} requirements for activation of muscle and the total amount of myofibrillar bound Ca^{2+} by using published and our own unpublished observations of the rates of exchange of Ca^{2+} with various muscle proteins. This was accomplished by calculating the time-course of the percent saturation of the Ca^{2+}-specific and the Ca^{2+}-Mg^{2+} sites of both cardiac and skeletal troponin, parvalbumin, calmodulin, and myosin with Ca^{2+} in response to a train of transient decreases in pCa. Each transient, denoted pCa(t), represents the time course of the free myoplasmic calcium ion concentration [Ca^{2+}] during a twitch. pCa(t) is quite different from, and should not be confused with, other time-dependent Ca^{2+} functions such as total cytostolic or total cellular Ca^{2+}. Nor is it a measure of the Ca^{2+} fluxes associated with the sarcoplasmic reticulum, mitochondria, or the sarcolemma during a twitch. Instead, pCa(t) is the net result of all the Ca^{2+} fluxes into and out of the myoplasm plus those associated with Ca^{2+} binding to all its various binding sites within the cell (i.e., proteins, phospholipids, etc.).

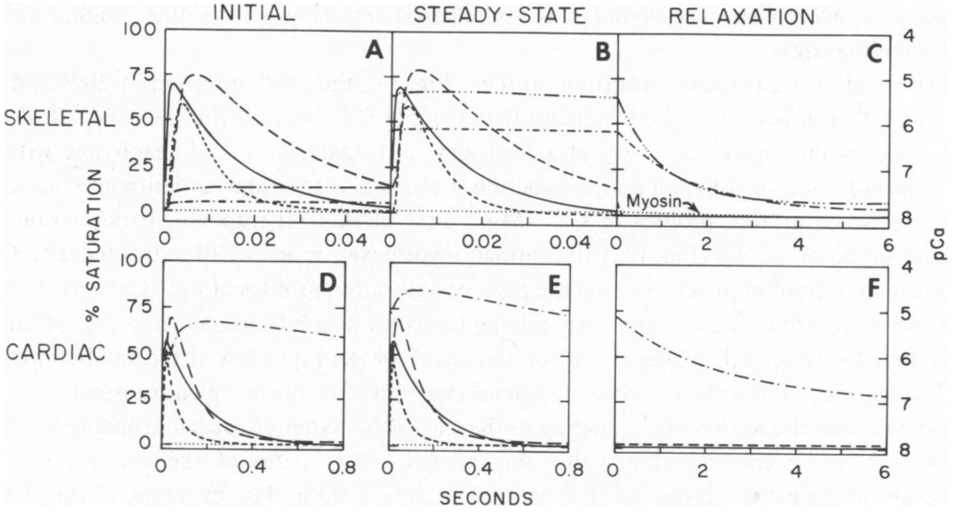

FIGURE 1 Percentage of maximal Ca^{2+} saturation of the indicated classes of Ca^{2+}-binding sites in response to pCa transients. The top three panels (A-C) correspond to the fast model in which the rate constants for Ca^{2+} and Mg^{2+} binding to the Ca^{2+}-Mg^{2+} and Ca^{2+}-specific classes of sites on skeletal troponin are used. *D–F* illustrate the results for the slow model with the use of Ca^{2+} and Mg^{2+} rate constants for cardiac troponin. pCa is represented by solid lines and the scale for this variable is shown on the far right of the figure. *A* and *D* illustrate the increase in the Ca^{2+}-saturation of the various classes of sites in response to a single pCa transient. Both transients started at pCa 8, and in the slow model Ca^{2+} peaked at 2×10^{-6}M (pCa 5.7) (*D–E*), while in the fast model Ca^{2+} reached a maximum of 6.3×10^{-6}M (pCa 5.2) (*A–B*). The steady-state percent-saturation profiles are shown in panels *B* and *E*. These were obtained by restarting the fast transients every 50 ms or the slow transients every 800 ms. The abscissa in *B* indicates time from the start of the 300th transient while that in *E* shows time from the start of the 15th transient. *C* and *F* show the slow rate at which Ca^{2+} dissociates from the Ca^{2+}-Mg^{2+} sites. At $t = 0$ in these panels pCa was set to 8.0 and the Ca^{2+} saturation levels of all the sites were set to their final values shown in panels *B* and *E*. ———, Ca^{2+}-specific; ----, calmodulin; —·—, Ca^{2+}-Mg^{2+}; —···—, parvalbumin; ····, myosin; —, pCa.

The percent saturation of all the Ca^{2+}-binding sites mentioned in Table I with Ca^{2+} resulting from trains of either fast (*A–B*) or slow (*D–E*) pCa transients are shown in Fig. 1. *A* and *D* illustrate the responses to the first transient of each train. The percent occupancy with Ca^{2+} of each class of sites corresponding to a resting pCa of 8 is shown at $t = 0$ in these two panels. The steady-state percent-saturation profiles in response to repetitive exposure to pCa transients are shown in Fig. 1*B* and *E* while *C* and *F* illustrate the way in which the models return to their prestimulated states when pCa is fixed at 8. Because skeletal actomysin ATPase appears to be regulated by Ca^{2+} binding to both of the Ca^{2+}-specific sites of skeletal troponin (10), we have plotted the expected percentage of skeletal troponin molecules that contain Ca^{2+} bound to both Ca^{2+}-specific sites in Fig. 1*A* and *B* while the percent occupancy of the single Ca^{2+}-specific site of cardiac troponin is shown in *D* and *E*.

The analyses presented in Fig. 1*A* and *D* clearly indicate that all the sites bind Ca^{2+} in response to a single pCa transient, but only the Ca-specific sites of troponin and calmodulin (the "Ca^{2+}-specific" type sites) become adequately saturated fast enough to play a role in rapid Ca^{2+} regulation of contraction. In contrast, the Ca^{2+} content of the Ca^{2+}-Mg^{2+} sites of troponin, parvalbumin, and myosin (the "Ca^{2+}-Mg^{2+}" type sites) increases by only a small percentage. Moreover, once bound, Ca^{2+} is released very slowly from these higher affinity Ca^{2+}-binding sites.

The steady-state percent-saturation profiles (Fig. 1*B*, the 300th transient at 20/s and Fig. 1*E* the 14th transient at 1.25/s) indicate that there is Ca^{2+} movement onto and off of each class of sites in response to every pCa transient. But again, only Ca^{2+} exchange with the Ca^{2+}-specific sites of troponin and calmodulin is analogous to a contraction-relaxation cycle. The percent-saturation profiles of Ca^{2+}-Mg^{2+} sites of skeletal troponin, parvalbumin, and myosin varied by <2.2% (Fig. 1*E*.) In contrast, myosin was never >2.3% saturated with Ca^{2+}. Following the train of pCa transients the percent-saturation profiles of the "Ca^{2+}-Mg^{2+}" type sites slowly return to their steady-state resting levels (at pCa 8.0) as shown in Fig. 1*C* and *F*. Note that the time scale of Fig. 1*C* is too slow to show the rapid loss of the small amount of Ca^{2+} remaining on the specific sites of skeletal troponin after the last pCa transient.

Even though the K_A for Mg^{2+} binding to the Ca^{2+}-Mg^{2+} sites of cardiac troponin is 17-fold smaller than the corresponding value for the Ca^{2+}-Mg^{2+} sites of skeletal troponin, the response of these two classes of sites is qualitatively similar. For example, if the skeletal instead of the cardiac troponin values were used in the slow model, the resting Ca^{2+} saturation of the Ca^{2+}-Mg^{2+} sites would be 5.6% as opposed to 26.1% for the cardiac sites. By the 12th transient the skeletal Ca^{2+}-Mg^{2+} sites would have achieved a steady-state profile ranging between 25 and 40% (the cardiac sites ranged between 72 and 85%). Thus, the response of the Ca^{2+}-Mg^{2+} sites of either skeletal or cardiac troponin to pCa(t) is too slow and too small to participate in the rapid Ca^{2+}-induced on-off regulation of muscle.

Inspection of Fig. 1*A*, *B*, *D*, and *E* reveals that, even though the response of the Ca^{2+}-Mg^{2+} sites to any single pCa transient is small, there is a gradual but substantial increase in the mean level of Ca^{2+} occupancy with repetitive exposure to pCa(t). This suggests that the percent-saturation profiles of the Ca^{2+}-Mg^{2+} sites of both troponins and that of parvalbumin represent integrals of pCa(t). In other words, their absolute values correspond more closely to a time average of pCa(t) rather than to pCa(t) itself, which implies that the percent saturation of these sites with Ca^{2+} would be a measure of the intensity and frequency of recent muscle activity. This type of response is a consequence of the millimolar myoplasmic [Mg^{2+}] and the slow rates at which both Ca^{2+} and Mg^{2+} dissociate from these sites. Initially (at pCa 8), 91, 99, and 97% of the Ca^{2+}-Mg^{2+} sites of cardiac and skeletal troponin and of parvalbumin, respectively, will contain divalent metal (mostly Mg^{2+} plus a small amount of Ca^{2+}). The small quick increase in the percent-saturation of these sites with Ca^{2+} occurring early in each response is due to Ca^{2+} binding rapidly to the metal-free sites (typically less than 5%). Any increase thereafter in the amount of Ca^{2+} bound results in and requires a corresponding decrease in the percent-saturation of these sites with Mg^{2+}. Thus, the apparent Ca^{2+} on-rate is essentially equal to the slow Mg^{2+} dissociation rate. In contrast, if [Mg^{2+}] were 10 μM, these sites initially would be about 75% saturated with divalent metal (mostly Ca^{2+} at pCa 8) and would fill to more than 93% with Ca^{2+} in response to the first pCa transient. Their steady-state profiles would therefore be reached more quickly and all peak near 100% Ca^{2+} bound (analysis not shown). After activity they would lose bound Ca^{2+} slowly as indicated in Fig. 1 *C* and *F* because of their high affinity for Ca^{2+}.

There is almost no pCa(t) induced Ca^{2+} binding to myosin because of its high affinity for Mg^{2+} and its lower affinity for Ca^{2+} (11, 12). Because at rest myosin is completely saturated with Mg^{2+}, all Ca^{2+} binding occurs slowly as it must exchange for bound Mg^{2+}. As a consequence, at the point of peak Ca^{2+} binding (Fig. 1 *B*) myosin contains only 10% of the Ca^{2+} it is capable of binding at a steady-state pCa of 5.2. [Mg^{2+}] must be reduced to about 10 μM before Ca^{2+} saturation in response to pCa(t) in the fast model exceeds 50% (analyses not shown). But even at 10 μM [Mg^{2+}], the total variation in the percent-saturation of myosin with Ca^{2+} during any single transient is still small, ~13% for the slow transients and ~6% for the fast transients.

The Ca^{2+} and Mg^{2+} on-rates for myosin calculated from the published off-rates and affinity constants (shown in Table I) are about 10-fold slower than those expected for diffusion-controlled reactions (28). Regardless of this fact, Ca^{2+} saturation of myosin never exceeds 1.7% at 2.5×10^{-3}M Mg^{2+} in response to either transient if on-rates of $10^8 M^{-1} s^{-1}$ and $10^5 M^{-1} s^{-1}$ and off-rates of 3.33 s^{-1} and 0.364 s^{-1} are used for Ca^{2+} and Mg^{2+}, respectively. The Ca^{2+}-binding responses of the Ca^{2+}-Mg^{2+} sites of myosin do, however, have about twice the amplitude, attain lower peak values, and reach their steady-state profiles quicker with the faster rate constants (analysis not shown).

Besides slowing dramatically the rate at which Ca^{2+} binds to the Ca^{2+}-Mg^{2+} sites of parvalbumin and those of skeletal and cardiac troponin, bound Mg^{2+} also induces changes in the conformation of these proteins which are qualitatively similar to those measured with Ca^{2+} bound to these sites (16, 19, 22, 23, 29).[1] Thus, if Ca^{2+} binding to these sites has regulatory significance, regulation occurs upon Ca^{2+}-Mg^{2+} exchange rather than upon Ca^{2+} binding to metal-free sites (22). Although the physiological role of Ca^{2+}-Mg^{2+} exchange at the "Ca^{2+}-Mg^{2+}" type sites is unknown, our analysis suggests that, whatever it may be, its response time is in terms of seconds, spanning many twitches. We are currently investigating the possibility that the Ca^{2+} exchange rates for the Ca^{2+}-specific sites of troponin may vary depending upon the relative amounts of Ca^{2+} and Mg^{2+} bound to its Ca^{2+}-Mg^{2+} sites. This

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[1] Potter, J. D., S. P. Robertson, F. Mandel, and J. D. Johnson. The kinetics of Ca^{2+} and Mg^{2+} exchange with carp parvalbumins: implication for the contraction-relaxation cycle. Submitted for publication.

may be a mechanism whereby changes in [Mg^{2+}] shift the pCa-force relationship (30). Conversely, Ca^{2+} binding to the Ca^{2+}-specific sites could alter the slow rate at which Ca^{2+} exchanges with Mg^{2+} bound to the Ca^{2+}-Mg^{2+} sites.

Fig. 2 indicates the time dependence of the total amount of Ca^{2+} bound by the Ca^{2+}-specific type and the Ca^{2+}-Mg^{2+} type sites in each model assuming the cellular contents of each protein as given in Table I. Although the parvalbumin content of fast-twitch muscles varies dramatically, we chose it to be equal to that of troponin, as it is in the rabbit (21), since our skeletal troponin rate constants were obtained from the rabbit. This figure demonstrates that the amount of Ca^{2+} required to activate skeletal muscle is significantly less than steady-state myofibrillar Ca^{2+}-binding data would suggest. For example, at the points of peak total Ca^{2+} bound in the fast model (Figs. 2 *A* or *B*) the Ca^{2+}-specific type sites contain ~82% of the total amount of Ca^{2+} they are capable of binding at a fixed pCa of 5.2. In contrast, the Ca^{2+}-Mg^{2+} type sites contain only 9% (Fig. 2 *A*) or 54% (Fig. 2 *B*) of the Ca^{2+} they could bind at the same steady-state pCa.

Note that >94% of the Ca^{2+} bound in response to any transient in the fast model or in response to the last transient in the slow model is bound to calmodulin and the Ca^{2+}-specific sites of troponin. Because both of these stimulation rates can produce trains of individual twitches in fast skeletal muscles, it is clear that only Ca^{2+} binding to the Ca^{2+}-specific type sites is fast enough to regulate force generation. The amount of Ca^{2+} required to activate muscle during each twitch is therefore determined primarily by the dynamic Ca^{2+}-binding capacity of the Ca^{2+}-specific type sites and not by the steady-state Ca^{2+}-binding capacity of all the classes of sites combined. Thus, the amount of Ca^{2+} required to activate muscle may be only one-half to one-third of that suggested by equilibrium Ca^{2+}-binding data.

Exact values, however, for the amount of Ca^{2+} required to activate muscle cannot be

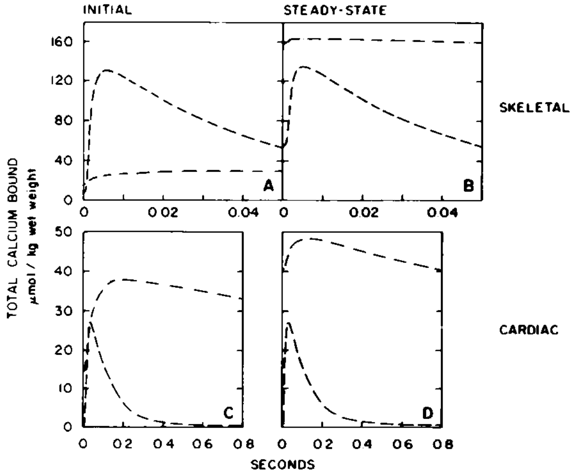

FIGURE 2 Total amount of Ca^{2+} bound and its distribution among the various classes of Ca^{2+}-binding sites as a function of time. — — —, total amount of Ca^{2+} bound by the "Ca^{2+}-specific" type sites during the course of the indicated transients; — · —, Ca^{2+} bound by the "Ca^{2+}-Mg^{2+}" type sites. The curves *A* and *B* were derived from Fig. 1 *A* and *B*, respectively, using the cellular content values for the skeletal proteins listed in Table I. The curves in *C* and *D* were derived from Fig. 1 *D* and *E*, respectively, using the cardiac cellular content values of Table I.

obtained from this analysis, because the pCa transients used in our models are not accurate descriptions of an in vivo [Ca^{2+}] transient. Furthermore, the temporal relationship between the percent-saturation of the Ca^{2+}-specific sites of troponin and the peak force developed during a twitch is unknown. But it is clear that during any twitch most of the calcium bound by the myofibrils is associated with Ca^{2+} binding to the "Ca^{2+}-specific" type sites (94, 95, 55, and 78% for Figs. 2 *A–D*, respectively).

Fig. 3 illustrates the time-course of Ca^{2+} saturation of the various Ca^{2+}-binding sites in the "skeletal" model in response to a step decrease in pCa from 8 to 5.2 in order to simulate a tetanus. Panels *A* and *B* contain similar data but are plotted with different time scales. These curves further demonstrate that the Ca^{2+}-specific type sites respond to pCa transients much more quickly than do the Ca^{2+}-Mg^{2+} type sites.

We have not included force records in our analysis even though the ultimate goal of this type of study is to correlate the time course of Ca^{2+} binding at each class of sites with force during a twitch. Exact temporal correlations can only be made when protein Ca^{2+}-binding properties, in vivo pCa transient measurement, and force records are obtained from the same muscles under similar conditions.

Our results suggest that Ca^{2+} binding to the Ca^{2+}-specific sites of both troponins may be too fast to account for all the time delay that exists between photometric signals which presumably report in vivo pCa transients and the subsequent force transients recorded in the same muscles (5–9, 24). A partial explanation for this discrepancy, which we are currently examining, is that the rates at which Ca^{2+}-induced conformational changes appear at various points along thin filaments (troponins I and T, tropomyosin and actin) may be slower than the rate at which Ca^{2+} binds to troponin. For example, Johnson et al. (31) have reported that the rate at which Ca^{2+}-induced structural changes appear in the TnI subunit of the troponin complex is considerably slower than the rate at which Ca^{2+} binds to troponin.

It is interesting to note that, although our results suggest that the Ca^{2+}-Mg^{2+} type sites probably do not play a direct role in the on-off regulation of muscles in vivo, these sites have been included in models describing Ca^{2+}-activated force generation in skinned skeletal muscle fibers in response to step changes in pCa(*t*) (32). It remains to be seen whether these sites are actually regulating force development or it is merely that their Ca^{2+}-binding kinetics are

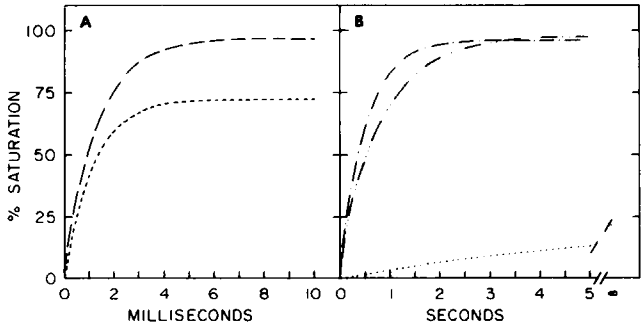

FIGURE 3 Amount of Ca^{2+} bound to each class of Ca^{2+}-binding sites as a function of time following a step change in pCa from 8 to 5.2. These curves represent the response of the skeletal proteins. Note the different time scale for the two panels. *A*, the "Ca^{2+}-specific" type site; *B*, the "Ca^{2+}-Mg^{2+}" type site. The line code for each class of sites is described in Fig. 1.

similar to the other processes that determine the time-course of force generation in these preparations.

Our calculations do not support the recent proposal of Haiech et al. (23) that parvalbumins act as rapid soluble "relaxing" sites in vivo. Fig. 1 shows that the amount of Ca^{2+} bound to parvalbumin during a twitch is very small compared with that bound to the Ca^{2+}-specific sites, and that once bound, Ca^{2+} dissociates too slowly to account for the rapid relaxation seen in muscles containing parvalbumin.

Finally, our suggestion that the amount of Ca^{2+} bound by these four major Ca^{2+}-binding proteins in active muscle is only about 33–50% of their steady-state Ca^{2+}-binding capacity, has several important implications. First, the amount of Ca^{2+} that must be handled by releasing and sequestration systems during each twitch is correspondingly reduced. In other words, the specific activity of the sarcoplasmic reticulum need not be as high as suggested by the steady-state myofibrillar Ca^{2+}-binding capacity. Another prediction is that following muscle activity, substantial Ca^{2+} sequestration occurs at low $[Ca^{2+}]$ long after force has subsided because of the slow rate at which Ca^{2+} dissociates from the Ca^{2+}-Mg^{2+} type sites (Fig. 1 *C* and *F*).

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