Mathematical Modeling of the Myosin Light Chain Kinase Activation

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Received May 1, 2005

The mathematical model presented here describes the interactions among Ca^{2+}, calmodulin (CaM), and myosin light chain kinase (MLCK) and consists of a kinetic scheme taking into account 7 reactions instead of 12 as proposed previously. We derive a system of 5 nonlinear ordinary differential equations. Solving it yields the prediction of active MLCK as a function of $[Ca^{2+}]$ whereby the active MLCK is defined to be proportional to the $Ca_4CaM{\cdot}MLCK$ complex concentration. The model predictions are compared with other theoretical and experimental predictions of active MLCK as well as with the results of our previously proposed complex model.

INTRODUCTION

Active Ca^{2+}/calmodulin dependent myosin light chain kinase (MLCK) has an important role in the process of myosin light chain (MLC) phosphorylation and consecutive smooth muscle contraction.[1,2] The generally accepted theory of smooth muscle contraction states that an increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]$) initiates the binding of four Ca^{2+} ions to four binding sites of calmodulin (CaM).[3] Then, according to this theory, Ca_4CaM complex interacts with MLCK and activates it.[3,4] The activated MLCK phosphorylates MLC which in turn results in a development of stress due to an increase in the actin-myosin cross bridge cycling rate.[5,6]

Although it is known that the affinities of the C- and N-terminal binding sites of CaM for Ca^{2+} are different, this theory ignores that property of CaM. Moreover, binding of Ca_2CaM complexes with MLCK is also not considered. Recently, this general view of interactions among Ca^{2+}, CaM, and MLCK has been improved as it has been experimentally demonstrated that not only Ca_4CaM but also Ca_2CaM complexes and even Ca^{2+}-free CaM can interact with and bind to MLCK.[7−9] Recent experimental results show that MLCK is present in a complex with Ca_2CaM in which Ca^{2+} is bound to the C-terminal of CaM at low $[Ca^{2+}]$.[9] It has been suggested that this is a consequence of the increased affinity of CaM for Ca^{2+} by the presence of MLCK in the complex.[9] In accordance with these findings the Ca^{2+}/CaM activation of MLCK appears at sufficiently high Ca^{2+} levels, when in addition to the C-terminal binding sites, the N-terminal binding sites for Ca^{2+} on CaM are saturated as well.[9]

In our recent paper[1] we presented a complex model of interactions between Ca^{2+}, CaM, and MLCK that considered binding of MLCK to Ca^{2+}-free CaM as well as to Ca_2CaM complexes. By changing the model parameters we simulated the MLCK phosphorylation and studied its effect on MLCK activation by Ca^{2+}/CaM. The proposed cubelike kinetic scheme involved 8 species, 12 reactions, and 24 rate constants. One of the unique aspects of the model was derivation of equilibrium constants related to the binding of MLCK to CaM with Ca^{2+} bound at either the C or N terminal lobes as well as the binding constants for Ca^{2+} to various intermediate Ca^{2+}/CaM−MLCK complexes.

Here we propose a mathematical model of a truncated complex kinetic scheme of interactions among Ca^{2+}, CaM, and MLCK introduced in previous paragraph.[1] Thus we avoid the need to predict unknown parameter values by model constraints. Also some experimental evidences speak in favor of the present kinetic scheme.[10] The present kinetic scheme omits two variables compared to the more complex one, whereby the number of parameter values reduces from 24 to 14 constants. The Wegscheider's condition is also applied as a model constraint retaining the parameter values coherent as previously proposed.[1] The study here is focused on the comparison among different model predictions, on the pros and cons with respect to reduction of the complex system, and, finally, on the temporal evolution of the system. Model predictions are compared with the recently published quantitative FRET (fluorescence resonance energy transfer) measurements of active MLCK in dependence on $[Ca^{2+}]$.[11]

MATERIALS AND METHODS

Kinetic Scheme. Mathematical modeling is used for analyzing the interactions among Ca^{2+}, CaM, and MLCK. The kinetic scheme presented in Figure 1 shows simultaneous binding of two Ca^{2+}-ions to each terminal of CaM. This approach can be justified by the difference in Ca^{2+} binding properties between N- and C-terminal binding sites of CaM and by the fact that the properties of Ca^{2+} binding to two binding sites within each terminal lobe are very similar.[12,13] In Figure 1 symbols CaM and M represent calmodulin and MLCK, respectively, the subscripts N and C represent two binding sites for Ca^{2+} at the N- and C-terminal of CaM, respectively. Each of them is occupied with a pair of Ca^{2+} ions. The subscript M represents the binding site on CaM occupied by MLCK, whereas an underscore (_) represents an unoccupied state at CaM for each of the above-mentioned binding sites. k_i and k_{-i} are the on- and off-rate constants,

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10.1021/ci050177i CCC: $30.25
Published on Web 10/15/2005

respectively. For example, the symbol $CaM_{\_CM}$ represents the $Ca_2CaM{\cdot}MLCK$ complex with two Ca^{2+} ions bound to the C terminal of CaM.

Model Equations. Considering three independent binding sites on CaM, each of which may be empty or occupied, yields 2^3 different species in the complete kinetic scheme. Such complete cubelike scheme was recently proposed, modeled, and analyzed.[1] That model uses 24 rate constants, a number significantly larger than 14 constants used here; however, there are only two variables omitted in the present model. These are $CaM_{\_\_M}$ and CaM_{N_M}. Existing knowledge of the CaM binding properties, i.e., very low affinity of Ca^{2+}-free CaM for MLCK,[14] and higher affinity of C-terminal binding sites than of N-terminal sites of CaM for Ca^{2+} as well as slow rate of some omitted reaction-steps speak in favor of a truncated version. Using it is, in our opinion, a valuable approach in building models as long as precise experimental data on the entire kinetic scheme are not readily obtainable. As shown in Figure 1 we take into account 6 different species. There are then 7 variables in the mathematical model: all different states of the CaM molecule represent six variables, and the free MLCK concentration represents one. $[Ca^{2+}]$ is considered as a parameter in the model. The time evolution of the system is described by the system of 5 ordinary differential eqs 1−5 and two algebraic eqs 6 and 7, the latter two corresponding to the conservation relations for CaM and MLCK concentrations, respectively. The equations are

$$\mathrm{d}[\mathrm{Ca}_{\_CM}]/\mathrm{d}t = -k_6[\mathrm{Ca}^{2+}]^2[\mathrm{Ca}_{\_CM}] + k_{-6}[\mathrm{CaM}_{NCM}] + k_5[\mathrm{M}][\mathrm{CaM}_{\_C\_}] - k_{-5}[\mathrm{CaM}_{\_CM}] \quad (1)$$

$$\mathrm{d}[\mathrm{Ca}_{NC\_}]/\mathrm{d}t = -k_7[\mathrm{M}][\mathrm{CaM}_{NC\_}] + k_{-7}[\mathrm{CaM}_{NCM}] + k_4[\mathrm{Ca}^{2+}]^2[\mathrm{CaM}_{\_C\_}] - k_{-4}[\mathrm{CaM}_{NC\_}] + k_3[\mathrm{Ca}^{2+}]^2[\mathrm{CaM}_{N\_\_}] - k_{-3}[\mathrm{CaM}_{NC\_}] \quad (2)$$

$$\mathrm{d}[\mathrm{CaM}_{\_C\_}]/\mathrm{d}t = -k_4[\mathrm{Ca}^{2+}]^2[\mathrm{CaM}_{\_C\_}] + k_{-4}[\mathrm{CaM}_{NC\_}] - k_5[\mathrm{M}][\mathrm{CaM}_{\_C\_}] + k_{-5}[\mathrm{CaM}_{\_CM}] + k_1[\mathrm{Ca}^{2+}]^2[\mathrm{CaM}_{\_\_\_}] - k_{-1}[\mathrm{CaM}_{\_C\_}] \quad (3)$$

$$\mathrm{d}[\mathrm{CaM}_{N\_\_}]/\mathrm{d}t = -k_3[\mathrm{Ca}^{2+}]^2[\mathrm{CaM}_{N\_\_}] + k_{-3}[\mathrm{CaM}_{NC\_}] + k_2[\mathrm{Ca}^{2+}]^2[\mathrm{CaM}_{\_\_\_}] - k_{-2}[\mathrm{CaM}_{N\_\_}] \quad (4)$$

$$\mathrm{d}[\mathrm{CaM}_{NCM}]/\mathrm{d}t = -k_{-6}[\mathrm{CaM}_{NCM}] + k_6[\mathrm{Ca}^{2+}]^2[\mathrm{CaM}_{\_CM}] + k_7[\mathrm{M}][\mathrm{CaM}_{NC\_}] - k_{-7}[\mathrm{CaM}_{NCM}] \quad (5)$$

$$[\mathrm{CaM}_{tot}] - ([\mathrm{CaM}_{\_\_\_}] + [\mathrm{CaM}_{NC\_}] + [\mathrm{CaM}_{\_CM}] + [\mathrm{CaM}_{\_C\_}] + [\mathrm{CaM}_{N\_\_}] + [\mathrm{CaM}_{NCM}]) = 0 \quad (6)$$

$$[\mathrm{M}_{tot}] - ([\mathrm{CaM}_{\_CM}] + [\mathrm{CaM}_{NCM}] + [\mathrm{M}]) = 0 \quad (7)$$

To compare the experimental findings with our model predictions we define the measure that quantifies active MLCK in dependence on $[Ca^{2+}]$, as previously proposed[1]

$$A = \frac{[\mathrm{CaM}_{NCM}]}{[\mathrm{M}_{tot}]} \quad (8)$$

which has values between 0 and 1.

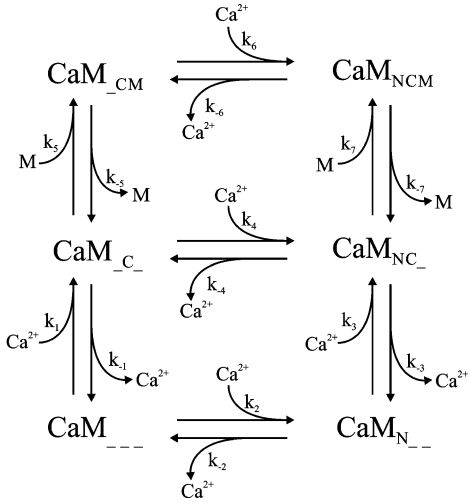

Figure 1. Kinetic scheme of interactions among Ca^{2+}, CaM, and MLCK. For symbols, see text.

Numerical integration of the system of eqs 1−7 was carried out with the stiff method in Berkeley Madonna 8.0 at the initial condition $[CaM\_\ \_\ \_] = [CaM_{tot}]$ for long enough time yielding the equilibrium states of the system variables with respect to different $[Ca^{2+}]$.

Model Parameters. Since we take into account the binding of two Ca^{2+} ions to CaM in our model, the on-rate constant is defined as $k_i = k_{-i}/K_i^2$, k_{-i} is the off-rate constant, and K_i is the dissociation constant. Therefore the relation between the model parameter k_i and experimental values of the apparent on-rate constant k_i^{app}, defined in the refs 8 and 15 as $k_i^{app} = k_{-i}/K_i$, is $k_i = k_i^{app}/K_i$. We apply the so-called detailed balance or Wegscheider's condition, which is valid in closed reaction systems,[16,17] as the model constraint

$$\prod_j K_j = 1 \quad (9)$$

where K_j denotes either K_i or K_i^2, depending on the reaction considered. K_i corresponds to the binding of MLCK to CaM and K_i^2 to the binding of two Ca^{2+} ions to CaM. For the subsequent analysis, we introduce the notation $K_i^2 = K_i'$. The same approach was used in our previous publication.[1]

The kinetic scheme in Figure 1 involves two closed cycles to which Wegscheider's condition can be applied. The equations describing them are as follows: $K_1'^{-1} K_2' K_3' K_4'^{-1}$, $K_5^{-1} K_7 K_4' K_6'^{-1}$ from which the parameters K_1' and K_5, respectively, are expressed as the unknown quantities, whereby only determination of K_5 gives additional information. This is a consequence of equal parameter values K_2 and K_4 which in turn implies $K_1 = K_3$.

The parameter values reflect the properties of smooth-muscle MLCK rather than of MLCK-peptide analogues or the skeletal- and nonmuscle MLCK isoforms. References consulted in obtaining the parameter values are listed in Table 1 along with the model parameters. It is worthwhile emphasizing that in some cases the values from the literature were not directly transferred into our model since other constraints were also taken into consideration. Thus, the parameters presented in Table 1 represent a coherent set of values obtained from the experimental data. Two parameter values were predicted using the Wegscheider's condition,

Table 1. Model Parameters

i	K_i [μM]	K_i' [μM^2]	k_i [μM^{-2} s^{-1}]	k_{-i} [s^{-1}]	refs
1	1.46	2.14[a,b]	2.80	6.00	8,12,14,15,22,26,27
2	2.83	8.00[a]	100	800	8,12,14,15,22,26,27
3	1.46	2.14[a]	2.80	6.00	8,12,14,15,22,26,27
4	2.83	8.00[a]	100	800	8,12,14,15,22,26,27
5	0.020[b]	/	1000	20	14
6	0.63	0.40[c]	12.5	5.0	8,14,20
7	0.0010[a]	/	1000	1.0	18−20

[a] Values taken the same as in ref 1. [b] Value calculated using Wegscheider's condition. [c] Value adjusted for the best fit to experimental data.

and one parameter has been adjusted for the best fit to experimental data (see Table 1). Furthermore, the following experimentally determined relationships were used in adjusting the parameter values used in the model. Namely, the K_d of smooth-muscle MLCK for Ca_4CaM is in the nM range,[18−20] whereas the K_d for Ca_2CaM is supposed to be approximately an order of magnitude higher.[14] The values used in the model are 1 nM (K_7) and 20 nM (K_5) for the MLCK affinity for Ca_4CaM and Ca_2CaM, respectively. The C-terminal affinity of free CaM for Ca^{2+} is approximately 1.5 μM and is according to the experimental results approximately 2−4 times higher than for the N-terminal.[8,21] The model values are 1.46 μM (K_1 and K_3) and 2.83 μM (K_2 and K_4) for the K_d values of the C- and N-terminal, respectively. According to the experimental results Ca^{2+} apparently binds approximately 70 times faster to the N-terminal than to the C-terminal.[8,22] In our model the ratio of apparent on-rate constants for reaction steps 1 and 2 as well as for 3 and 4 is $k_2^{app}/k_1^{app} = k_4^{app}/k_3^{app}$. Additionally, experimental results suggest that the dissociation of Ca^{2+} from the N-terminal of CaM is 140−225 times slower from the Ca_4CaM·MLCK complex than from the Ca_4CaM sole.[8] In our model the ratio of the off-rate constants k_{-4} and k_{-6} is 160.

For the total CaM concentration ([CaM_{tot}]) and the total MLCK concentration ([$MLCK_{tot}$]) we use the values 10 μM and 2 μM, respectively, which is in accordance with the concentrations measured in smooth muscles[23−25] and with the experiments predicting the MLCK activity.[11]

RESULTS AND DISCUSSION

Ca^{2+}/CaM dependent activation of MLCK has been studied in terms of mathematical modeling only in a few cases. In 1984 Kato et al. proposed a two-step kinetic model among Ca^{2+}, CaM, and MLCK.[28] Their approach was based on the assumption that only when Ca^{2+} occupied all four binding sites on CaM, this Ca_4CaM complex could bind to and activate MLCK. Moreover, they assumed four independent, equivalent Ca^{2+} binding sites on CaM. Authors Rembold and Murphy[29] predicted semitheoretically [Ca^{2+}] dependent activation of MLCK by finding a unique relationship between experimentally measured dependency of MLC phosphorylation on [Ca^{2+}] in vitro and the theoretically predicted MLCK activity. Recently, Geguchadze et al.[11] have quantitatively examined the activation of MLCK in vitro by a fluorescent biosensor MLCK where a Ca^{2+}-dependent increase in kinase activity was coincidental with decrease in FRET due to the Ca^{2+}/CaM binding to MLCK.[11] Observing a [Ca^{2+}] dependence of FRET signal similar to MLC phosphorylation, FRET was not suggested only as a measure of relative Ca_4CaM·MLCK concentration but as a measure of MLCK activity as well.[11] Additionally, these data are in agreement with data on Ca^{2+} dependent MLCK activity published in ref 30.

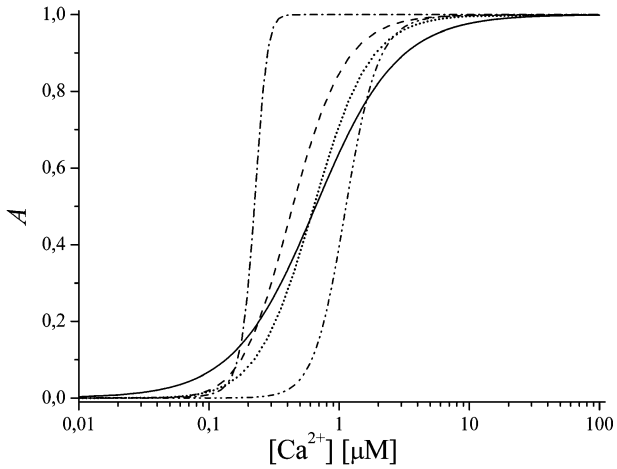

Figure 2. Relative amount of active MLCK (A) versus [Ca^{2+}]. Hill coefficients (n) and half saturation values ($K_{0.5}$) of corresponding interpolated Hill functions are presented in brackets next to the curve notation. Fit to experimental data of ref 11 (solid line, n=1.4, $K_{0.5}$=0.66 μM); prediction of the truncated model (dotted line, n=2.06, $K_{0.5}$=0.640 μM); prediction of the complex model of ref 1 (dashed line, n=2.27, $K_{0.5}$=0.444 μM); semitheoretical prediction of ref 29 (dash−dot line, n=5.3, $K_{0.5}$=0.25 μM); and model prediction of ref 28 (dash−dot-dot line, n=3.95, $K_{0.5}$=1.04 μM.

In Figure 2 we compare predictions of a truncated model for active MLCK versus [Ca^{2+}], defined as A, with the model predictions of the refs 28 and 29, with experimental predictions of ref 11 as well as with predictions of a previously reported complex model.[1] The data from the ref 11 were obtained by the use of the Digitizer tool in Origin 6.1 and were afterward fitted in the same program with the Hill function of the form $[Ca^{2+}]^n/(K_{0.5}^n + [Ca^{2+}]^n)$. The same action was performed with all model predictions. The characteristic coefficients of the fits are presented in the captions of Figure 2. The dotted and the dashed lines represent model predictions of truncated and complex models,[1] respectively, whereas the dash−dot and dash−dot−dot lines represent the semitheoretical prediction of Rembold and Murphy[29] and the prediction of Kato et al.,[28] respectively. The prediction of Rembold and Murphy differs significantly from all other predictions. On the other hand, the predictions of Kato et al. and our model results are in reasonable agreement but indicating a noticeably different steepness. Note that Hill coefficients (n) of the fitted Hill function differ approximately 2-fold (see captions of Figure 2). Greater steepness of the model prediction of Kato et al. is a consequence of the fact that the authors did not distinguish between different rates and properties of Ca^{2+} binding to the two terminals of CaM, they neglected that Ca_2CaM complexes could bind to MLCK, and they used the fourth-order mass-action kinetics in their modeling. Our models, previous complex and the present truncated, give relatively similar predictions with respect to equilibrium data. The recently published data for active MLCK versus [Ca^{2+}] in the ref 11 evidently speak in favor of our model predictions; however, the steepness of the experimental prediction is

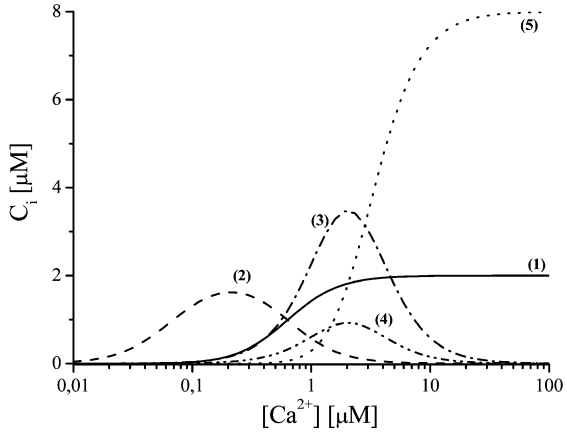

Figure 3. The steady-state concentration of the *i*th CaM species (C_i) versus [Ca^{2+}]: (1) CaM_{NCM}; (2) $CaM_{\_CM}$; (3) $CaM_{C\_}$; (4) $CaM_{N\_\ \_}$; and (5) $CaM_{NC\_}$.

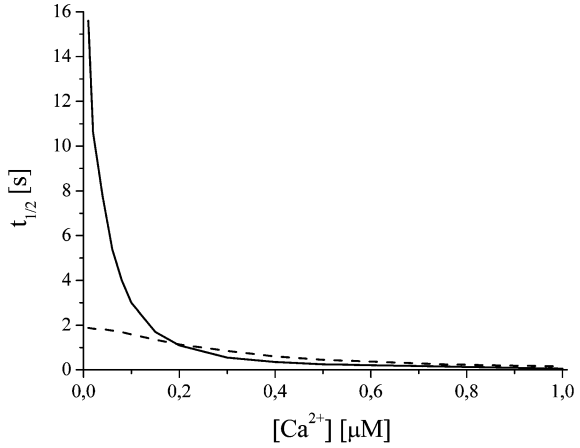

Figure 4. The half saturation time ($t_{1/2}$) of the model variable *A* versus [Ca^{2+}] predicted by truncated model (solid line) and complex model[1] (dashed line).

lower (n=1.4). The disagreement between experimental and our model predictions could be due to the fact that only 15 experimental data points were available for fitting the Hill function or due to the fact that it is not completely clear whether the experimentally obtained data of the FRET signal are the measure of the active specimen, i.e., $Ca_4CaM{\cdot}MLCK$, solely or of $Ca_2CaM{\cdot}MLCK$ complexes, as well.[11] Smaller steepness with the Hill coefficients around 1.8 and 1.1 is observed when fitting the predictions of the complex and the truncated model (curves not explicitly shown), respectively, by considering $Ca_2CaM{\cdot}MLCK$ complexes in the definition of *A*. It is also worthwhile emphasizing that the model curves in our previous work[1] were not fitted directly to the experimental data of ref 11. They emerged following the adjustments of the model parameter values within the reasonable range of known experimental values and depending on the constraints such as the Wegscheider's condition and many ratios between certain parameter values.[1] In the present work the parameter value K_6 was adjusted for the best fit to the experimental data; however, changing it influenced the parameter $K_{0.5}$ of the Hill function, solely.

The present model is capable of predicting non-negligible [$CaM_{\_CM}$] at low [Ca^{2+}], as proposed in ref 9. Despite omitting the $CaM_{\_\ \_M}$ species in the present model, this truncated model predicts a similar result as a more complex one.[1] This is visible from Figure 3 that represents concentrations of five CaM species versus [Ca^{2+}]. Note that the dashed curve (2) representing the $Ca_2CaM{\cdot}MLCK$ complex concentration ([$CaM_{\_CM}$]) is significantly high at low [Ca^{2+}]. This is in agreement with the previously proposed view that under this condition a higher affinity of the C-terminal of CaM for Ca^{2+} is responsible for Ca^{2+} binding to CaM and for the subsequent binding of this Ca_2CaM complex to MLCK without activating it.[7] On the other hand, binding of Ca^{2+} to the N-terminal is postulated to provide the rapid response in transition from inactive $Ca_2CaM{\cdot}MLCK$ to active $Ca_4CaM{\cdot}MLCK$ complex at higher [Ca^{2+}].[7−9] At a very high saturating concentration Ca^{2+} is bound to all four binding sites of CaM and finally the Ca_4CaM complex interacts with MLCK, almost exclusively.

Further comparison between both our models is performed on the time evolution of the dynamical system. The equilibrium analysis was presented previously; however, it was pointed out that the dynamical analysis might be also of great importance. This is due to the fact that the characteristic time of the model variable *A* needed to reach the equilibrium (especially in the range of physiologically significant [Ca^{2+}]) is similar to the characteristic time of Ca^{2+} transients in cytosolic Ca^{2+} oscillations or nonoscillatory Ca^{2+} signals observed in smooth muscle cells.[31] Figure 4 shows the half saturation time ($t_{1/2}$) of the model variable *A* versus [Ca^{2+}] for two model predictions: the solid line for truncated model and the dashed line for the complex model.[1] The diagram shows a significant difference especially at low [Ca^{2+}]. This difference probably arises from the fact that Ca^{2+}-free CaM−MLCK complex, which concentration is significantly high at very low [Ca^{2+}] (data from the complex model not explicitly shown) and to which Ca^{2+} is binding with very high affinity albeit slowly, is missing in the present model. Although differences in equilibrium data are not noticeably expressed, the difference in temporal evolution especially at low physiological [Ca^{2+}] is evident.

CONCLUSIONS

In summary, the general simple view that after an increase in [Ca^{2+}], Ca^{2+} ions bind to four binding sites of CaM, and that only the Ca_4CaM complex interacts with and activates MLCK, was recently improved and broadened by the fact that also Ca_2CaM species, likely that with occupied C-terminal, can interact with MLCK.[9] Recent experimental findings also suggest much different rates and affinities for Ca^{2+} binding to CaM when CaM is associated with MLCK, namely, the N-terminal dissociation rate for Ca^{2+} is decreased by the factor more than 100[8,14] in such a case. Following these new findings we recently modeled mathematically a novel complex kinetic scheme of interactions among Ca^{2+}, CaM, and MLCK.[1] The analysis of truncated model indicates the importance of omitted intermediates especially from the point of view of temporal evolution of the complex system. Further comparison shows that our models represent an improvement in the modeling of interactions between Ca^{2+}, CaM, and MLCK with respect to other models; however, a complete agreement between theoretical model and experiment has not been achieved yet. Both our models predict relatively slow temporal evolution of the active MLCK, especially at low physiological [Ca^{2+}]. Thus, rapid equilibrium approximation will not be useful when Ca^{2+} transients would be incorporated into the model.

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CI050177I