Development of a Novel Markov Chain Model for Oxidative-dependent CaMKIIδ Activation

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Abstract

Dysfunction in the Calcium (Ca^{2+})-calmodulin (CaM) dependent kinase II (CaMKII) signalling can lead to several pathologies, such as heart failure and arrhythmia. Especially, the role of CaMKII signalling in oxidative stress-induced arrhythmias remains unclear. In this study, we aimed to develop a new Markov chain model of CaMKII δ-isoform (CaMKIIδ) that involves both of the autophosphorylation and oxidation pathways to better simulate CaMKII signalling under oxidative stress in cardiomyocytes. Based on the four-state model developed by Chiba et al., we implemented two oxidized states including a Ca^{2+}/CaM-bound state and a Ca^{2+}/CaM-dissociated state, representing the new pathway of oxidation-dependent activation. Using the model, we reproduced the CaM affinity to CaMKIIδ, the dependence of autophosphorylation on CaM. The frequency-dependent activation of CaMKII was simulated for both CaMKII α- and δ-isoforms. For the oxidation pathway, our simulation suggested that H_2O_2 increased the kinase activity in a dose-dependent manner, which also fitted to experimental data. Finally this model was incorporated in a human atrial cell model to simulate the effects of CaMKII activation on cellular action potentials.

1. Introduction

Ca^{2+}/CaM dependent protein kinase II (CaMKII) plays a key role in connecting upstream cellular signals to cellular behaviours. Dysfunction of CaMKII under reactive oxygen species (ROS) has been found in many pathologies, including heart failure, apoptosis, hypertrophy, and myocardium infarction. Recently, new findings [1] on the oxidative activation pathway of CaMKII further expands our horizons in understanding the mechanism responsible for dysfunctional CaMKII-induced heart diseases.

It has been shown that oxidized CaMKII can trigger atrial fibrillation [2]. However, it is incompletely understood yet the role of oxidative CaMKII in the genesis of atrial arrhythmias.

In this study, we (1) developed a novel CaMKII Markov chain model that considered the oxidative activation pathway; (2) fitted parameters of this model to better reproduce the experimental data compared with other studies; (3) incorporated this CaMKII model into a human atrial cell model to investigate its impact on human atrial action potential.

2. Methods

2.1. Model structure

A CaMKII holoenzyme is usually assembled by 8-14 CaMKII subunits. A single CaMKII has 3 domains named the association domain, the regulatory domain, and the catalytic domain. Binding of a CaMCa4 (a CaM binding with 4 Ca^{2+}) to the regulatory domain exposes the catalytic domain, which means its activation (state CaMKII_CaMCa4 in Figure 1). Under this state and in the presence of ATP, CaMKII can be further autophosphorylated, and then gets long lasting activity even upon dissociation of CaMCa4. In addition, recent study [1] has pointed out that CaMKII under the

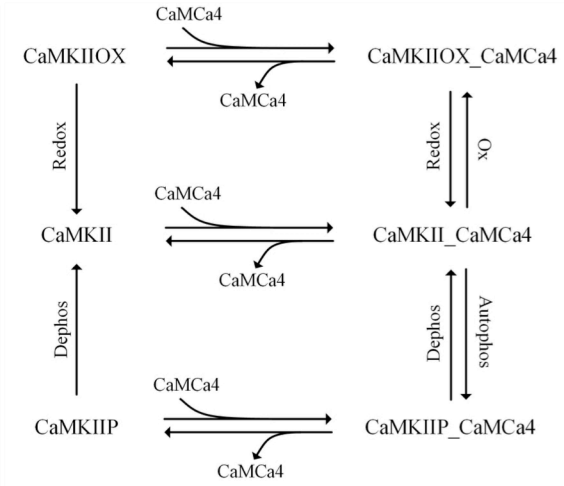

Figure 1. A schematic illustration of our CaMKII model. There is only one inactive state (CaMKII) in this model, other states including three Ca^{2+}/CaM bounding states and two phosphorylated or oxidative states are all active.

CaMKII_CaMCa4 state can be oxidized by ROS, which is similar to autophosphorylation. CaMKII is completely deactivated only after dephosphorylation by protein phosphatases (PP), or reduction by methionine sulfoxide reductases (MsrA). Since their study did not reveal any interaction between the autophosphorylation and oxidation pathway, we hypothesized that these two processes cannot happen at the same time.

Based on this hypothesis and the 4-state Markov chain model developed by Chiba *et al.* [3], we constructed a 6-state model in which an oxidized CaMCa4-bound state and an oxidized CaMCa4-unbound state were added (state CaMKIIOX and CaMKIIOX_CaMCa4 in Figure 1). These 2 states imitate the conformational change of CaMKII under oxidative stress.

It seems that oxidation and autophosphorylation of CaMKII share the same properties, in fact, however, their behaviors are different. First, the affinity of phosphorylated CaMKII to CaMCa4 is 1000 folds higher than primitive CaMKII. But in the oxidative pathway, this difference doesn't exist. In other word, oxidative CaMKII does not have the "CaM trapping" ability. Second, we know that autophosphorylation occurs between two neighbouring CaMKII subunits. However, it is unclear whether oxidation happens in the same way as autophosphorylation. In our model construction, we hypothesize that oxidation arises on a single subunit.

2.2. Cell Model

We modified the human atrial cell model developed by Grandi *et al.* [4] by incorporating our CaMKII model into its framework.

With respect to how CaMKII affects ion channels, we adopted a formulation developed by O'Hara *et al.* [5]. In this formulation, the population of a certain type of channel is divided into two groups; one phosphorylated by CaMKII, the other not. The total current is the sum of them. Then there are two questions need to be answered. (1) Given the percentage of activated CaMKII, how many channels will be phosphorylated? (2) If a channel is phosphorylated by CaMKII, what changes to it shall be made?

For the first question, we first calculate the fraction of activated CaMKII (CaMKII_active) as ($[CaMKII]_{total}$-$[CaMKII]$)/$[CaMKII]_{total}$. Then the fraction of phosphorylated channels is:

$$frac_p = \frac{1}{1 + \frac{K_{m,CaMKII}}{CaMKII_{active}}}$$

Where $K_{m,CaMKII}$ is a constant describing the affinity of CaMKII to a type of channel.

For the second question, on the basis of experimental data, we modified equations of currents by changing gates or multiplied current densities by coefficients. For sodium channel I_{Na}, we slowed the time constant of gate j by 1.46-fold, which controls the recovery from inactivation [6]. I_{CaL} was increased by 10% and the time constant of gate f was slowed down by 2.5-fold [7]. RyR calcium release and leak currents were increased by 25% [8], and the SR Ca^{2+}-ATPase current was increased by 75% [9].

3. Results

3.1. CaMKIIδ model without oxidation

When oxidation of CaMKII is absent, our model is generally the same as the Chiba model [3]. However, we noticed that they fitted their model manually and the

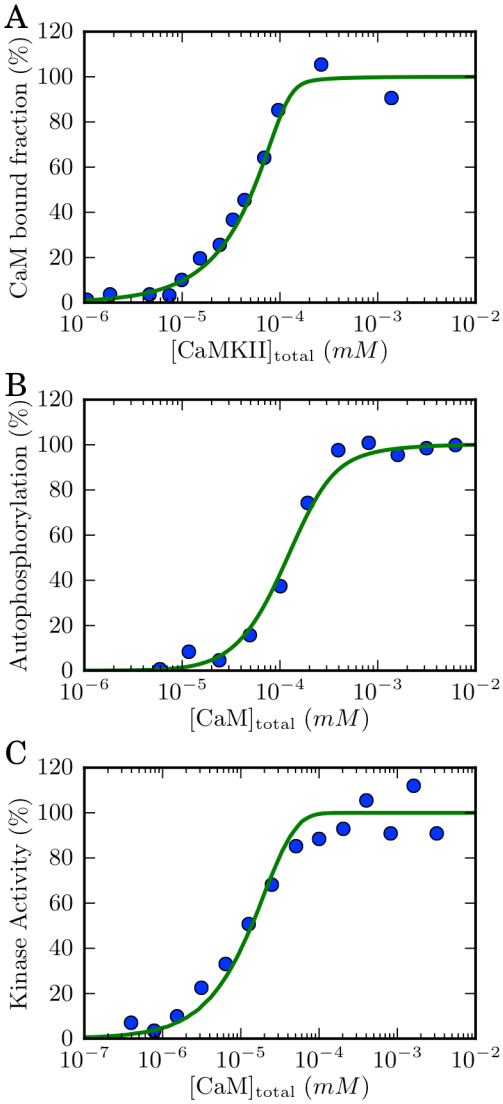

Figure 2. Simulated results (solid line) compared to experimental data (circles) from Gaertner *et al.* (A) Steady-state CaM bounding fraction to CaMKIIδ. (B) Autophosphorylation under certain amount of ATP. (C) Dependence of CaMKII activity on CaM.

parameters they obtained cannot well reproduce a series of experimental data describing CaMKII's kinase activity. So we tried to refit parameters in our model using identical conditions in simulation compared with experiments. In Figure 2A, results were obtained by fixing the [Ca^{2+}] to 0.5mM, adding 0.1μM [CaM], and then running the model for 60 seconds. Bounding fraction of CaM was calculated as (1-[CaMKII_CaMCa4])/[CaM]_total. Because there was no ATP or ROS added in this experiment, no autophosphorylation or oxidation occurred. Figure 2B shows the level of CaMKII autophosphorylation after incubating 62nM CaMKII in 100μM ATP, 0.5mM Ca^{2+} and different amount of CaM (1-10,000nM). Since the ratio of autophosphorylated CaMKII was low, we plotted the percentage of autophosphorylated CaMKII normalized to maximum level of autophosphorylation, which corresponded to the experimental condition. Figure 2C demonstrates the kinase activity of CaMKII in about the same condition as 2B, except the temperature was 30℃ rather than 0℃ and the reaction time was 60s rather than 15s. Kinase activity was normalized to the maximum value of it, because the exact value of kinase activity could not be obtained *in silico*. Our simulation results well reproduced these three series of experimental data.

3.2. Frequency-dependent activation

CaMKII plays an important role in regulating the Ca^{2+} handling in cardiomyocytes, especially at high heart rates. Aiming to investigate the frequency-dependent characteristic of autophosphorylated CaMKII, we simulated the autophosphorylation level of CaMKII under periodic stimuli. In the study of De Koninck *et al*. [10], experiment was conducted in the equipment that could cyclically expose CaMKII to a controlled solution containing 0.5mM Ca^{2+}, 100nM CaM and 0.25mM ATP. For example, the 1Hz data was attained by treating CaMKII with the solution 200ms per second, and then the level of autophosphorylation at different timings was measured. In simulations, the model for CaMKIIα with refitted parameters well reproduced the experimental data of De Koninck *et al.* [10]. In the experimental study of Gaertner *et al*. [11], it was found that the affinity of CaMKIIδ to CaM was higher than that of CaMKIIα, and so did the autophosphorylation rate. Our simulation results agreed with their data, showing that CaMKIIδ had a higher level of autophosphorylation at various frequencies.

3.3. Oxidative-dependent Activation

Erickson *et al.* [1] found that CaMKII can be activated by ROS in a dose dependent manner. In the experiment, they first treated CaMKII with Ca^{2+}/CaM and H_2O_2, then

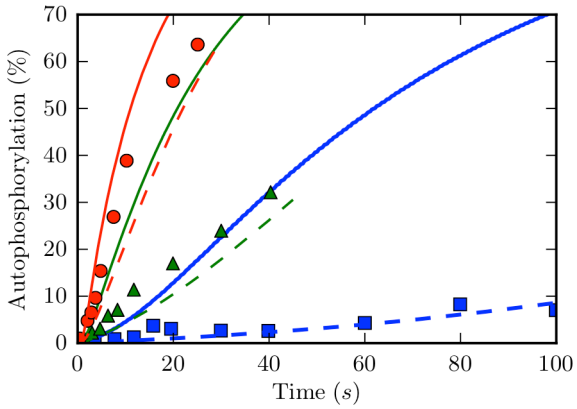

Figure 3. Frequency-dependent activation of CaMKIIα and CaMKIIδ. Three series of simulation and experimental results are represented by their color, 1Hz (blue), 2.5Hz (green), and 4Hz (red). Circles, triangles and squares are experimental data of CaMKIIα from De Koninck *et al*. Dashed lines are simulation results for CaMKIIα. Solid lines are simulation results for CaMKIIδ.

added EGTA to sequester Ca^{2+}. After these steps, the activation of CaMKII bounding with the Ca^{2+}/CaM was blocked. The remaining activity observed should be of oxidative CaMKII (CaMKIIOX).

In order to determine the oxidative rate, we redid the experiment of Erickson *et al.* [1] *in silico*, and then fitted the result curve to their data (Figure 4). The kinase activity showed in Figure 4 was normalized to the maximum value of it.

To our best knowledge, there is no experiment measuring the reductive rate constant of MsrA on oxidative CaMKII. Instead, we found there is literature [12] assessing the enzyme activity of MsrA on another substrate. The reported Michaelis-Menten constant (K_m) of MsrA was 0.34mM, and the turnover number (k_{cat}) of it was 0.28. We used these parameters in our model.

3.4. CaMKII and atrial cell model

The simulation results of CaMKII incorporated atrial

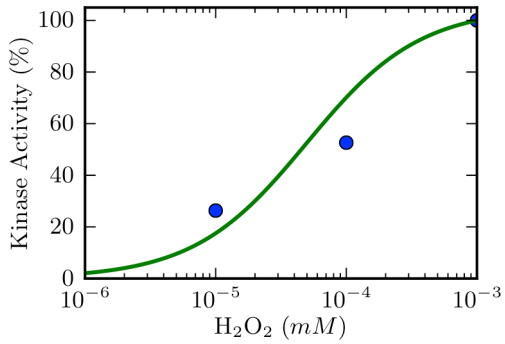

Figure 4. Fitted model data (line) to experimental data of kinase activity of CaMKIIδ by oxidative activation.

cell model are shown in Figure 5. We stimulated the model at a fixed rate (3.3 and 1 Hz respectively) for 60s, and then recorded the last AP. The percentage of activated CaMKII was about 23.3% and 5.3% in the 300ms- and 1000ms-PCL simulation, respectively. We did not plot the time trace of CaMKII because the change of it in a single AP was not obvious. It was shown in Figure 5, as the pacing rate increased, RyR release and $[Ca^{2+}]_{junc}$ dramatically rose, the inactivation of I_{CaL} slowed down and then led to a higher plateau phase in AP. But due to the offset effect of other currents, AP duration did not change noticeably.

4. Conclusion

In this study, a novel Markov chain model has been developed to reproduce both autophosphorylation-dependent and oxidation-dependent CaMKII activation. We also integrated it into an existing atrial electro-physiological model, which may be a powerful tool to explore the role of irregular functional CaMKII under oxidative stress.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (NSFC) under Grant No. 61179009, No. 61173086.

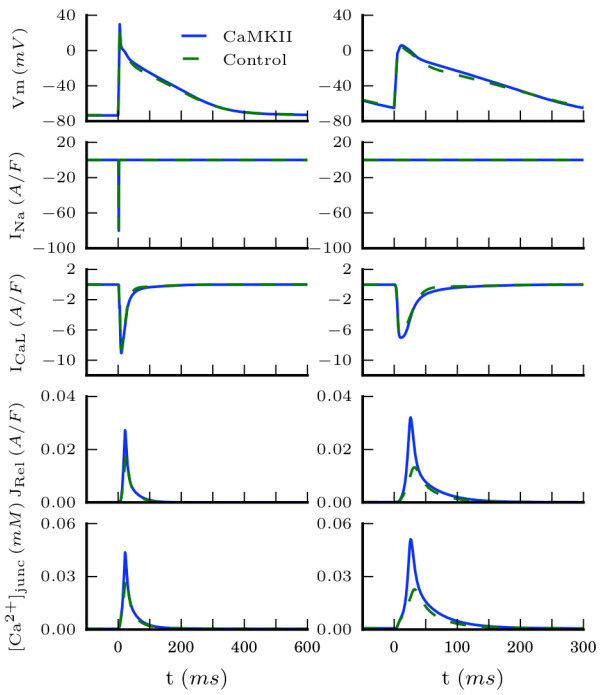

Figure 5. Action potential, major modified currents, and Ca^{2+} concentration at junctional cleft of the control model and CaMKII incorporated model. Left panels were pacing at 1s PCL, right panels at 300ms.

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