STIM1-ORAI1 direct interaction cannot govern store-operated calcium entry (SOCE) in platelets

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Abstract

Store-operated calcium entry (SOCE) plays an important role in platelet function. It is generally assumed that the mechanism of SOCE relies on the direct interaction of STIM1 and ORAI1 proteins with specific STIM1:ORAI1 stoichiometry. However, in platelets, other pathways may take place. Here we aim to investigate the mechanisms of SOCE in platelets. We developed a lattice-based mathematical model that represented STIM1-ORAI1 interactions and applied it to both HEK cells, where SOCE mechanism is well established, and platelets. The model was able to describe STIM1-ORAI1 behavior in HEK cells successfully. We used the same parameters for protein interaction and applied them to platelets. As a result, we demonstrated that the number of STIM1 proteins on ER membrane could not assure the needed stoichiometry to proper SOCE in platelets.

Keywords: Platelet intracellular signaling, Store-Operated Calcium Entry, Mathematical Modeling, Platelets

Introduction

Platelets play an essential role in hemostasis as they prevent blood loss upon vessel wall disruption [1]. This is achieved by their activation, subsequent aggregation and thrombus formation. It is well known that these processes are mainly governed by the dynamics of intracellular Ca^{2+} concentration [2]. There are two main sources of free Ca^{2+} that provide elevation of this ion in the cytosol after activation: intracellular stores (represented by endoplasmic reticulum (ER)) and extracellular medium [3], [4]. Major extracellular Ca^{2+} influx pathway in platelets is store-operated calcium entry (SOCE), that is caused by store depletion.

Two main proteins contributing to SOCE are ER protein STIM1, that senses Ca^{2+} levels in intracellular stores [5] and plasma membrane (PM) protein ORAI1, that forms Ca^{2+} highly selective channel [6].

STIM1 and ORAI1 in platelets contribute to thrombus formation, procoagulant activity and GPVI activation [7].

What is the mechanism of SOCE in platelets? While the crucial role of STIM1 and ORAI1 proteins is well established [8], [9], their interplay leading to Ca^{2+} influx in platelets is still unclear. The main mechanism of SOCE is considered to be STIM1-ORAI1 direct interaction and conformational coupling [6], [10], [11]. This was shown for many cells in vitro, specially on HEK293 cell line [12]–[14], where many aspects of STIM1-ORAI1 interaction were studied in detail. The chain of events is following: 1) ER depletion leads to dissociation of Ca^{2+} ions from STIM1 EF-hand motif, 2) this induces conformational changes in whole protein and its transition to an extended state, 3) activated STIM1 can couple with ORAI1 protein in ER-PM junctions (form puncta) if the spacing between two membranes is ~15 nm, 4) ORAI1 interaction with STIM1 opens the hexameric pore and significantly enhances Ca^{2+} conductance. Another interesting feature of coupling mechanism is the stoichiometry that leads to SOCE. It seems interaction of one STIM1 protein with pore is not sufficient for proper pore opening and at least 5 STIM1 are needed to induce Ca^{2+} influx (for more detail see review [15]).

Besides direct STIM1-ORAI1 interaction there is an alternative SOCE mechanism. It requires intermediate diffusible messenger – calcium influx factor (CIF) [16], the nature of which is still unknown. Store depletion and subsequent STIM1 activation leads to production of CIF [17] which activates calcium-independent phospholipase A2 (iPLA2) by displacing inhibitory calmodulin from its active site. Activated iPLA₂, in turn, leads to pore opening and, thus, Ca^{2+} influx. ORAI1 opening is considered to be associated with the presence of lysophospholipids, that are products of iPLA2 activity. This mechanism does not imply direct STIM1-ORAI1 coupling, however protein proximity is needed for rapid diffusion of CIF from ER to PM.

There are several mathematical models of SOCE [18]–[20]. Mass-action based model proposed

by Schmidt et. al. [20] considers processes of oligomerization, diffusion and clustering of STIM1-ORAI1. Despite the fact that this model takes into account the ER-PM junctions, specifically the diffusion of proteins in these regions, the modeling principle assumes a large number of molecules. Indeed, this approach is valid for large cells with a large number of proteins of interest (for example, HEK, which were used to validate the data). However, this cannot be applied to cells with significantly smaller number of proteins, as the system can no longer be considered as homogeneous. Considering relatively low STIM1 counts in platelet it is reasonable to use different type of approach for SOCE modelling. One of this approaches can be lattice-based models [21], where the system is represented by a mesh of cells that have a finite number of states. Each cell evolves according to a specific set of rules that represents temporal evolution of the system.

In this article we propose a lattice-based model of SOCE in platelets. We demonstrate that small surface concentration of STIM1 cannot provide sufficient stoichiometry for proper pore opening and Ca^{2+} influx.

Methods

Modelling Approach

The SOCE model is defined by two discrete spatial lattices $\mathcal{L}$: ER and PM membranes, a discrete state space ε and local rule-based dynamics.

The regular lattice $\mathcal{L} \subset \mathbb{R}^2$ consists of N nodes $r_i \in \mathcal{L}$, $i = 1, \dots, N$. Every node has $b = 8$ nearest neighbors. Each lattice node $r_i \in \mathcal{L}$ is connected to its nearest neighbor by unit vectors c_j , $j = 1, \dots, b$. The neighborhood is assumed to be Moore neighborhood. In addition, protein can be in a resting state, that is presented as c_0 . As we consider two opposing lattices,

that denote two membranes (ER and PM), additional unit vector c_9 is introduced in order to represent the occupation in the second lattice at same lattice cell. The parameter $K = b + 1$ defines the possible node movement choices.

The state space is defined through the occupation numbers $s_j \in \{0, 1\}$, $j = 0, \dots, K$. These occupation numbers represent the presence ($s_j = 1$) or absence ($s_j = 0$) of a protein in the channel c_j within some node. Then, the configuration of a node is given by the state vector:

$$s = (s_1, \dots, s_K) \in \varepsilon = \{0, 1\}^K.$$

The node by itself has properties. In our model STIM1(ORAI1) can be in two states – free, bound. Additionally STIM1 can be a part of a cluster. This fact is denoted by the parameter $a \in \{0, 1, 2\}$, where $a = 0$ represents free protein, $a = 1$ – bound, $a = 2$ – in cluster (for STIM1). If a protein is in bound state it can no longer diffuse along the membrane (Fig. 1).

A new lattice configuration is created according to a local rule that determines the new state of each node in terms of the current states of the node and the nodes in its neighborhood. In order to determine a new lattice configuration, the local rule is applied sequentially at every node r of the lattice.

The algorithm, by which STIM1-ORAI1 interaction was modelled is presented in the following block-diagram (Fig.2).

Note that we assume that close location of proteins is not sufficient for clustering and they need to “overlap” in order to form a cluster. Therefore, we consider clustering as a process that occurs when one protein tends to diffuse “into” another protein, rather than cluster due to simple proximity.

Initial state of each membrane is produced by randomly placing nodes on 2-dimensional lattice. The number of nodes for each lattice is equal to the

Figure 1. Scheme of single STIM1-ORAI1 interaction within the model. STIM1 protein is represented by black color, ORAI1 – grey. Diffusion of proteins along their membranes leads to their interaction and subsequent coupling.

Figure 2. Block-diagram representing modelling algorithm.

number of proteins (STIM1 or ORAI1).

Model parameters were assessed manually in order to match experimental data. Specifically, STIM1-ORAI1 probability was estimated according to known characteristic times and maximal number of STIM1-ORAI1 complexes and STIM1 clustering according to known STIM1:ORAI1 stoichiometry within one puncta.

Results

Evaluation of diffusion coefficient

Our modelling approach for diffusion is similar to modelling Brownian motion using a random walk. However, length of the step for each iteration is fixed and is equal to 1 lattice cell. This is due to the fact that when simulating the Brownian motion of particles in water, the collision length of a particle is much larger than the size of the particle itself due to the liquid state of aggregation of the medium [22]. When modeling diffusion along the membrane, the density of lipids and membrane proteins makes it possible to neglect this parameter. Fig.3 shows, that our approach and parameters for diffusion modelling gives us similar diffusion rate [13] and typical MSD(time) dependencies.

STIM1-ORAI1 direct interaction in HEK cells

In order to investigate the mechanism of SOCE in platelets, we first applied our model on HEK cells. Such approach was chosen based on two reasons: 1) HEK cells have well known geometric parameters and protein counts, including typical PM-ER junction areas, 2) STIM1-ORAI1 direct interaction is mostly investigated on this type of cells and so there is plenty of experimental data, on which model can be evaluated. The parameters for HEK cells were taken from [20] and are presented in Table 1.

Typical system state for ER membrane on $t = 0$ ms, 10 ms and 100 ms is shown in Fig. 4A. The model parameters were estimated to match rapid STIM1-ORAI1 puncta formation ([20], [23]) and then

subsequent cluster growth. Clustering probability does not significantly affect system dynamics (typical characteristic times remain in same order) (Fig. 4B). For much lower diffusion coefficient ($0.01 \mu\text{m}^2/\text{s}$) resulting dynamics does not change (Fig. 4B, dashed lines). As a result, clustering does not affect the total number of puncta, but contribute to stoichiometry and therefore correct pore opening (Fig. 4C).

Table 1.

Cell	HEK	Platelet
Area (μm^2)	530	70
ER-PM contact site total area (μm^2)	80	unknown ~25
Single contact area (μm^2)	~25-30	unknown
#STIM1	240000	7400
#ORAI1	120000	3700
Probability of STIM1- ORAI1 puncta formation due interaction	0.1-90%	

SOCE modelling in platelets

After estimating parameters for STIM1-ORAI1 interaction we applied our model on platelets. We used same probabilities for clustering and binding, but changed the initial number of proteins on each membrane. It appears, that such low STIM1 concentration cannot provide normal stoichiometry. Typical system states are presented in Fig.5A. It also seems that characteristic time of f STIM1-ORAI1 puncta formation is enhanced (Fig. 5B).

Discussion

In the present work we developed a lattice-based model of STIM1-ORAI1 interaction. The model was able to adequately describe protein coupling dynamics in HEK cells, which was in accordance to experimental data and results of different modelling approach. Our data also revealed that in HEK cells crosslinking of ORAI1 pores by STIM1 takes place, that according to [24] can change pore conductance properties.

$$D = 0.21 \mu\text{m}^2/\text{s}$$

Figure 3. Diffusion of single protein along the lattice. Blue color represents MSD of $n=100$ proteins. Orange color shows individual SD.

Figure 4. (A) STIM1-ORAI1 interaction for HEK cells. Bound proteins are presented by brown, STIM1 in cluster – green, free proteins - red. (B) STIM1-ORAI1 puncta formation dependance on clustering probability. Dashed lines represent same process for $D = 0.01 \mu\text{m}^2/\text{s}$ (C) Typical system state at $t=100\text{ms}$ for different clustering probabilities

On the other side, in platelets not only crosslinking does not occur, but neither needed stoichiometry is achieved. This is due to small surface STIM1 concentration, even if all STIM1 proteins are considered to be fully on ER, although they are presented both on PM and ER.

There are several ways to interpret the obtained data. Firstly, we may assume that direct interaction does not play significant role in platelets. Then, as STIM1 and ORAI1 are known to mediate platelet function, we consider different mechanism of SOCE. iPLA2 pathway of SOCE is known to be present in platelets and may be responsible for SOCE instead of direct coupling model. However, it was shown by Harper et. al. [25] that iPLA2 activation is not sufficient for SOCE activation in platelets, which is still discussible.

Another possible mechanism of SOCE still accounts direct interaction of STIM1 and ORAI1, but with addition of TRPC channels [26], [27]. It is known that TRPC1 play role in SOCE and also can be activated by coupling with STIM1. In addition, there is evidence that TRP channels tend to colocalize with ORAI pores. Thus, ORAI1-TRPC complex may react different to activation of STIM1 and not require such

Figure 5. (A) STIM1-ORAI1 interaction for platelets. Bound proteins are presented by brown, STIM1 in cluster – green, free proteins - red. (B) STIM1-ORAI1 puncta formation dependance on clustering probability

stoichiometry as ORAI1 solely.

Author Contributions

Conceptualization, A.N.S. and A.K.G.D.; methodology, A.K.G.D.; software, A.K.G.D.; validation, A.K.G.D.; investigation, A.K.G.D.; resources, A.N.S.; data curation, A.N.S.; writing—original draft preparation, A.K.G.D.; writing—review and editing A.N.S.; visualization, A.K.G.D.; supervision, A.N.S.; project administration, A.N.S.; funding acquisition, A.N.S. All authors have read and agreed to the published version of the manuscript.

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