J Comput Aided Mol Des (2013) 27:697–705
DOI 10.1007/s10822-013-9668-0

Combining fragment homology modeling with molecular dynamics aims at prediction of Ca^{2+} binding sites in CaBPs

ChunLi Pang · TianGuang Cao · JunWei Li · MengWen Jia · SuHua Zhang · ShuXi Ren · HaiLong An · Yong Zhan

Received: 8 April 2013 / Accepted: 31 July 2013 / Published online: 10 August 2013

Abstract The family of calcium-binding proteins (CaBPs) consists of dozens of members and contributes to all aspects of the cell's function, from homeostasis to learning and memory. However, the Ca^{2+}-binding mechanism is still unclear for most of CaBPs. To identify the Ca^{2+}-binding sites of CaBPs, this study presented a computational approach which combined the fragment homology modeling with molecular dynamics simulation. For validation, we performed a two-step strategy as follows: first, the approach is used to identify the Ca^{2+}-binding sites of CaBPs, which have the EF-hand Ca^{2+}-binding site and the detailed binding mechanism. To accomplish this, eighteen crystal structures of CaBPs with 49 Ca^{2+}-binding sites are selected to be analyzed including calmodulin. The computational method identified 43 from 49 Ca^{2+}-binding sites. Second, we performed the approach to large-conductance Ca^{2+}-activated K^+ (BK) channels which don't have clear Ca^{2+}-binding mechanism. The simulated results are consistent with the experimental data. The computational approach may shed some light on the identification of Ca^{2+}-binding sites in CaBPs.

Keywords Fragment homology modeling (FHM) · Molecular dynamics (MD) simulation · Ca^{2+}-binding proteins (CaBPs) · Ca^{2+}-binding site

Electronic supplementary material The online version of this article (doi:10.1007/s10822-013-9668-0) contains supplementary material, which is available to authorized users.

C. Pang · T. Cao · J. Li · M. Jia · S. Zhang · S. Ren · H. An · Y. Zhan (✉)
Institute of Biophysics, Hebei University of Technology, Tianjin, China
e-mail: hebut_zhanyong@hotmail.com

Introduction

Calcium (Ca^{2+}) is the most common second messenger and plays a central role in the function of many types of cell. The functions of Ca^{2+}, such as muscle contraction, blood vessel contraction and expansion, secretion of hormones, and modulation of neuron activity [1–4] must be mediated by Ca^{2+}-binding proteins (CaBPs). There are dozens of CaBPs which are involved in many physiological processes. For example, some CaBPs can bind Ca^{2+} and activate enzymes or initiate a series of steps that lead to muscle contraction [5–7], which include troponin, parvalbumin, and calsequestrin, and the most ubiquitous one is calmodulin (CaM), which has been found in all eukaryotic organisms including yeasts. Other CaBPs involve in cell signaling and metabolism, such as calexcitin, calbindin and S100 protein [8–10]. There are also Ca^{2+} sensitive ion channels, such as large-conductance Ca^{2+}-activated K^+ (BK) channels [11] and Ca^{2+}-activated chloride channels (CaCCs) [12]. Ca^{2+} and the CaBPs have been recognized to take part in all aspects of cell function, starting with a cell's birth during mitosis and ending with its apoptotic death.

To understand the detailed mechanism of Ca^{2+} modulating CaBPs, identification of Ca^{2+}-binding site is one of the most concerned questions. The calcium ions are stabilized at the binding sites which are formed with several oxygen atoms from water molecules and carboxyl of special amino acids [13, 14]. The growing database of crystal structures of the CaBPs reveals that there is a great diversity of conformations, domain organization, and structure responses to Ca^{2+}. The EF-hand, C2 domain and Ca^{2+} bowl appear often in the Ca^{2+}-binding or Ca^{2+}-activated proteins [15–17], almost all of these sites include a dozen residues, the critical residues also named binding sites are always Glu or Asp or other residue which provide carbonyl oxygen atoms. Mutagenesis and crystallogoraphy are used to identify the Ca^{2+}-binding sites.

However, very few CaBPs have X-ray crystallographic structures among the whole superfamily of CaBPs. Moreover, it is impossible to search all amino acids with site-direct mutation (SDM) to determine the binding site. It is quite difficult to identify the Ca^{2+}-binding site in CaBPs which has neither crystal structure nor adequate SDM data, such as Ca^{2+}-activated chloride channel [18].

Here we performed a computational approach which combined the fragment homology modeling (FHM) with molecular dynamic (MD) simulation aimed at identification of the Ca^{2+}-binding site in CaBPs without crystal structures. For validation, a two-step strategy is performed which is started with classical MD simulation. In the first step, eighteen structures were extracted from protein data bank (PDB) protein database which contain 49 Ca^{2+}-binding sites, and 18 MD runs were carried out for each structure. Compared with the crystal structures, there are 43 binding sites and 150 binding residues from 49 to 211, respectively, are identified. Next, we moved to identify the Ca^{2+}-binding sites of BK channel which has relatively adequate SDM data but not the crystal structure. The FHM and MD simulation are performed and Ca^{2+} are binding at D367 (known as Ca^{2+}-binding residue) [19] and D369 (a novel residue) in RCK1 domain and D895, D898 and D900 (all known as Ca^{2+} biding residues) in RCK2 domain [20].

Simulation methods

Fragment homology modeling

The primary sequence of BK channel is obtained from the SwissProt database (accession number Q28265.2). Two fragments are selected to be modeled. The first one relates to the Ca^{2+}-binding site in the RCK1, and the second one relates to the binding site of the Ca^{2+} bowl, each fragment contains about 30 amino acids (aas). To help define the 3D structure of the two fragments, the secondary structure prediction is employed by using online APSSP Server (http://imtech.res.in/raghava/apssp/).

In homology modeling, BLAST (Basic Local Alignment Search Tool) at NCBI website was used to find templates. The crystal structures of calbindin D28 K (2F33) and human Vigilin (2CTL) are obtained from the PDB as the modeling templates of the two fragments of BK channel, respectively. To align the sequence of BK channel fragments with that of the templates, the clustalW plugin of VMD was employed [21].

MODELLER version 9v7 [22] was used to build homology models of the two fragments from their crystallographic template structure. An ensemble of 5 model structures containing all non-hydrogen atoms was generated. From the 5 models, the structure corresponding to the lowest value of DOPE score (Discrete Optimized Protein Energy) was selected for further analysis and as a starting structure for the MD simulations. The stereochemistry of the selected structure has also been validated by the Procheck program [23].

Molecular dynamics simulation

Ca^{2+}, no less than twice the number of binding sites, was added into the solvated box of the simulation system. Accordingly, there are about 10 Ca^{2+} in each EF hand or C2 domain protein, and 2 Ca^{2+} in the BK fragments. Chloride ions are additionally added in the system to neutralize the residual charge. Mead ionize, a plugin of VMD, was used to add the ions into the minima of the electrostatic potential map of the solvated system by replacing water molecules. The electrostatic potential was generated by the 'potential' utility of the MEAD (Macroscopic Electrostatics with Atomic Detail) program package [24, 25].

The complex which included protein, water and manually added ions are used as the starting structure for nanosecond MD simulation. All of the simulations are completed by NAMD2.7 (www.ks.uiuc.edu/Research/namd/) [26] using CHARMM27 force-field [27]. Every MD runs involved more than 10 thousands of atoms and requires about 2 days on a 16 CPU system. Every simulation is carried out in three phases: an initial period of energy minimization in NVT ensemble for 1 ps, and a consequently energy equilibrium in NPT ensemble for 2 ps (MD simulation in NPT ensemble is finished until the stabilization of pressure), and a governing phase of energy equilibrium in NVT ensemble for 10 ns.

The simulation finished here also with the following characteristics: ① Lennard-Jones (L–J) interactions were calculated with a cutoff distance of 12 Å and a smooth function is employed for the vdW interaction at a distance of 10 Å; ② Periodic boundary conditions are introduced to stabilize the simulation space; ③ The temperature is maintained 310 K by means of Langevin's algorithm; ④ Particle-Mesh Ewald technique are applied in our water-box system to treat the long-range electrostatic potential; ⑤ All of bonds are believed flexible and the time step is 1 fs; ⑥ The C_α in the middle of the backbone is fixed to prevent the protein's extending out of the water box [26, 28].

Visualization and analysis of model features is carried out using VMD1.8.

Results

MD Simulation identified 43 from 49 Ca^{2+}-binding sites in CaBPs with known structures

The EF-hand motif and C2 domain is the most common Ca^{2+}-binding domain which form suitable conformation

and stabilize Ca^{2+} in it. The classical EF-hand is a helix-loop-helix motif characterized by a sequence of, usually, 12 residues that starts with an aspartate and ends with a glutamate [29]. The typical version of C2 domain has a beta-sandwich composed of 8 β-strands that coordinates two or three Ca^{2+}, which bind in a cavity formed by the first and final loops of the domain [15]. All of these Ca^{2+}-binding sites are rich in acidic amino acids and provide four to eight oxygen atoms to binding the Ca^{2+}. To date, there are nearly 400 EF-hand protein structures, and tens of thousands of hints that are related to the C2 domains to date. The idea is that MD simulation can match Ca^{2+} and the binding sites of CaBPs based on the minimum energy principle. For validation, the authors selected 18 structures from 7 types CaBPs which contains 49 Ca^{2+}-binding sites and performed 10 ns MD simulations on each structure. Our data shown that the MD simulation identified the 43 from 49 Ca^{2+}-binding sites of CaBPs.

Calcium ions rush to and stay in the EF-hands of CaM during MD

Calmodulin, found in all eukaryotic organisms including yeasts, is a small (∼17 kD), ubiquitous, highly conserved EF-hand containing Ca^{2+}-binding protein. The crystal structure shows that CaM is a dumb-bell-shaped molecule, with two similar lobes connected by a central alpha-helix and contains two Ca^{2+}-binding EF-hand loops in each lobe [30]. To evaluate whether the MD simulation can identify the Ca^{2+}-binding sites of CaBPs, we start with a MD simulation performed on the CaM N-terminal domain [31]. We took the Ca^{2+} in the crystal structure away and placed 0.1 mM Ca^{2+} into the solvated system followed the principle of minimum potential energy. After 10 ns MD simulation, the Ca ions are stabilized at the EF-hand Ca^{2+}-binding sites.

During the process of the simulation, the C_α RMSD of the protein is used to evaluate the overall stability of system and the success of the simulation; Stepwise rising of RMSD correlates with the gradual approaching to the stable state of the protein, reaching a nearly plateau of 3 Å within 2 ns of the energy equilibrium simulation, suggesting that a 10-ns simulation is sufficient for assessing the stable binding of ions to protein. We compare the stable simulated state with the crystal state of ions-protein complex and found that they have much in common. The identified binding site is the EF-hand loop which plausibly acts as Ca^{2+}-binding site in the structure (Fig. 1a, b).

More in detail, the MD simulation analysis concerned with the dynamic profile of distance between Ca^{2+} and

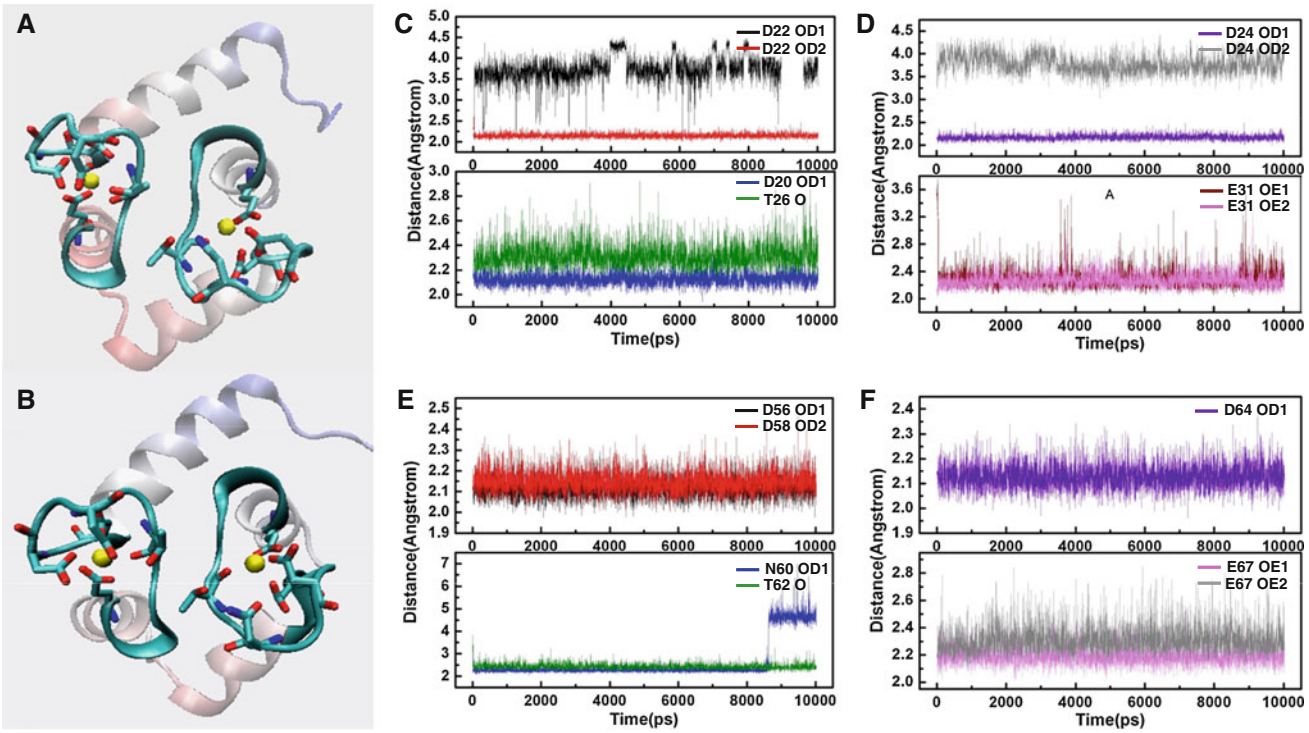

Fig. 1 MD confirmed the Ca^{2+}-binding sites in N terminal domain of CaM. **a**, **b** show the crystal structure and computational result respectively, the EF-hand sites are highlighted as *cyan, yellow spheres* represent Ca^{2+}. **c**–**f** are the evolution of distance between bound Ca^{2+} and the carbonyl oxygen atoms of the residue come from the two EF-hands respectively. Different *colors* represent the distance between calcium ions and corresponding oxygen atom

Table 1 The binding sites and the ligands of the Ca^{2+} in the N terminal domain of CaM

EF-hand	1		2	
Residues forming the H–L–H structure	6–19, 29–39 20–31		45–55, 65–75 56–67	
	Crystal result	MD result	Crystal result	MD result
Metal ligands/the distance (A)	D20 OD1/2.59	D20 OD1/2.10	D56 OD2/2.59	D56 OD2/2.10
	D22 OD1/2.64	D22 OD1/3.80	D58 OD1/2.58	D58 OD1/4.11
	D22 OD2/2.59	D22 OD2/2.15	D58 OD2/3.00	D58 OD2/2.10
	D24 OD1/2.59	D24 OD1/2.15	N60 OD1/2.58	N60 OD1/2.52
	T26 O/2.59	T26 O/2.37	T62 O/2.58	T62 O/2.40
	E31 OE1/2.59	E31 OE1/2.35	E67 OE1/2.60	E67 OE1/2.24
	E31 OE2/2.59	E31 OE2/2.24	E67 OE2/2.60	E67 OE2/2.34
				D64 OD2/2.15

Crystal result indicates the data from the crystal structure of CaM N terminal domain with PDB code :1J7O; MD result indicates the data from the stable state of simulation; The distance indicates the distance between the ligand oxygen atom with the bound $Ca^{2}+$

Table 2 Summary of MD simulation in confirming the Ca^{2+}-binding sites of CaBPs

	Structure	Binding site	Binding residue	Binding oxygen
Crystal result	18	49	211	264
MD result	18	43	150	189
Accuracy		87.8 %	71.1 %	71.6 %

ligand oxygen atom provided by the residues located in EF-hand, which is considered suggestive of the binding interactions (Fig. 1c–f). Two Ca ions reached the corresponding sites of EF-hand and stay there for relative long time. Each Ca^{2+} is coordinated by six to seven oxygen atoms mainly come from carbonyl of residues. The responsible residues binding the Ca^{2+} are just the members of EF-hand site. As distance profile shows (Fig. 1c, d), the first simulated binging site is from the Asp 20 to Glu31 in EF-hand 1 of CaM. The second simulated binding site is the Asp56, Asp58, Asn60, Thr62 and Glu67 in EF-hand 2 (Fig. 1e, f). In the process of 10 ns of the simulation, the distance between Ca^{2+} and the ligand oxygen atoms of related residue reached the minimum of 2 Å and the maximum of 4 Å (Table 1).

MD efficiently determines the Ca^{2+}-binding sites in CaBPs

In order to validate the efficiency of MD in making out the binding site of CaBPs, we analyzed eighteen crystal structures with the same MD procedure used in the CaM N terminal domain above. All of the selected structures are in their Ca^{2+}-saturated states as this would be beneficial for later comparison. These representative structures are selected from different kind of EF-hand proteins and C2 domain proteins, e.g. calmodulin, troponin, parvalbumin, calbindin, calexcitin, S100 and protein kinase C (alpha type), Rabphilin-3A, as shown in the supporting materials. They suggested that the Ca^{2+} affinity of the CaBPs is determined by both the acid–base property of a few residues (mainly 5) and the 3D structure of the fragment (mainly loop sandwiched by two helices or beta sheets).

As our work concerned, the summary data is shown in Table 2. MD simulation can not make out the Ca^{2+}-binding sites in all structures; there are 49 binding sites in the 18 structures, and the working efficiency of MD is 87.8 %. All the identified binding sites are not exactly the same as the crystal results, 211 residues attending in binding Ca^{2+} in the crystal structures, and 150 of them are identified. As for the carbonyl oxygen, the accuracy of the prediction is 71.6 %. Since the two carbonyl oxygen atoms are equivalent to each other, so we have not differentiated them in the simulation. From the statistics point view, the result indicates a considerable probability of prediction. In the condition of having obtained the structure and without knowing the binding site of the CaBPs, the theoretical prediction could help us to find the binding site more quickly and more economically.

Combining the fragment homology modeling (FHM) and MD simulation to confirm the Ca^{2+}-binding sites in BK channel

BK channels are widespread in neurons, skeletal and smooth muscles and other tissues. Although the most important physiological property is dual regulation through membrane voltage and intracellular Ca^{2+}, there is no typical Ca^{2+}-binding site as we discussed above in it. A large number of SDM studies about the Ca^{2+} affinity of BK channel were carried out [32–34], which aid the evaluation

of membrane protein modeling and simulation methodology. Gradually, two putative high affinity Ca^{2+}-binding sites in the cytosolic domain arose: one locates in the RCK1 (regulator of K^+ conductance) domain at position D362/D367 and the other one in a region termed Ca^{2+} bowl which contains a series of Asp residues in the RCK2 domain [11, 32]. There have been several computational studies concerned with the Ca^{2+} bowl which explained the SDM results of this site and made some descriptions of the binding molecular mechanisms [17, 35]. Yet, there rarely are the simulation studies about the description of D362/D367 Ca^{2+}-binding site. Crystal structures of the gating ring of BK channel both in Ca^{2+}-free and Ca^{2+}-bound states present a molecular basis for Ca^{2+} activation of K^+ channels [20, 36, 37]. However, the crystal structures didn't tell the detailed characteristics of all the Ca^{2+}-binding sites, for example, which residue is most critical in binding Ca^{2+} and how the Ca^{2+} is coordinated by oxygen atoms? The knowledge of Ca^{2+}-binding site in BK channel is far less than the understanding of EF-hand.

Based on the effectiveness test of MD simulation in finding Ca^{2+}-binding site of CaBPs with known structure carried out before, we execute the methodology of combination of the FHM and MD simulation to give a description of the Ca^{2+}-binding sites in BK channel. Two fragments are selected which contain several tens of amino acids to test our idea, the first one is concerned with D362/D367 binding site, and the second one is concerned with the Ca^{2+} bowl.

FHM and MD simulation predicted the Ca^{2+}-binding site in RCK1 of BK channel

We started to construct a model for Ca^{2+}-binding sites in RCK1 domain. The templates are found followed by two principles, high sequence identity and similar physiological functions. The structure of Ca^{2+}-loaded calbindin D28 K (PDB ID: 2F33) can be employed as a template for it. Figure 2 shows the alignment of amino acid sequences for fragment 1 and its template, and the sequence identity is 46 %. Moreover, both BK channels and cabining belong to CaBPs superfamily. Based on the sequence alignment, the modeled structure appeared to be helix, and the critical residues locate in the middle and final of it respectively.

Fig. 2 Sequence alignment of fragment 1 with its template and the secondary structure prediction of it. The residues are colored in similarity. PSSP is abbreviation for protein secondary structure prediction

The model is favorable with the prediction of the secondary structure of the fragment.

After constructing the 3D structure of the first fragment, MD simulation is performed to investigate the conformational variations of the fragment within the hydrated environment. The crystal structure of fragment 1 is shown in Fig. 3a, and the stable simulated state included the bound Ca^{2+} is shown in Fig. 3b. The distance evolution (Fig. 3c, d) indicates that the ions get to the destinations and are tightly bound by the special residues. The first Ca^{2+} is bound by D362 and the second one is bound by D367 and D369. There are several acidic residues in the fragment and there are several Ca^{2+} in the solvent, only the D362 and D367 binding Ca^{2+} respectively, which indicated that these two residues are most sensible to Ca^{2+}.

FHM and MD validated the binding site in Ca^{2+} bowl

For the second fragment, the template is identified with the high sequence identity and similarity to the predicted secondary structure. The best model of the Ca^{2+} bowl produced by Modeller is based on the solution structure of the 13th KH type I domain from human Vigilin (PDB ID: 2CTL). Figure 4 shows the sequence alignment between the target and the template, and the identity between them is 38 %. The produced model appears mainly to be loop, and the five continuous aspartates come to semi-circular. The model is checked by Modeller and Procheck, and is reliable for further MD simulation studies.

Figure 5a shows the crystal structure of Ca^{2+} bowl in the gating ring of BK channel, and Fig. 5b shows the stable state of the modeled structure, both in Ca^{2+} loaded manner. The distance evolution between Ca^{2+} and Asp895/Asp898, as well as Asp900, is shown in Fig. 5c, d. During the simulation, Ca^{2+} reaches in the binding sites and is stabilized by the special aspartates. The distance between Ca^{2+} and the carbonyl oxygen atom of the D895/D898/D900 is about 2.3 Å. As speculated before Ca^{2+} bowl form a binding loop with D899 at the center of the turn, its side chain extending away from the Ca^{2+}, and on the other side, D895/D898 and D900 extending their side chains inward to coordinate Ca^{2+}. As a result, the five Asp in Ca^{2+} bowl are not equally important in binding Ca^{2+}, D898 and D900 is more critical than others both from the experimental and theoretical point of view.

Discussion

The interaction between Ca^{2+} and CaBPs, or the prediction of Ca^{2+}-binding site in CaBPs is always an open problem [38]. More and more CaBPs are discovered with diverse binding site,which promote the investigation of new

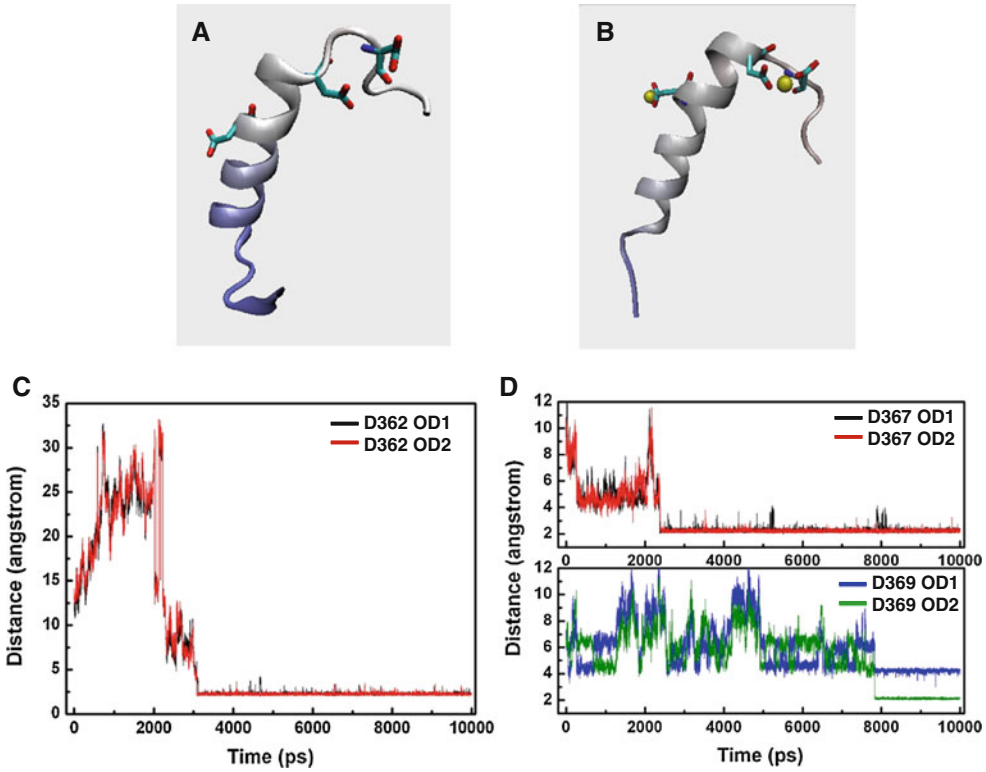

Fig. 3 FHM and MD simulation predicted the Ca^{2+}-binding residues in fragment 1 of BK channel. **a** Stereoview of the crystal structure of fragment 1. **b** FHM models the structure of fragment 1 with Ca^{2+} loaded by MD. D362, D367, D369 are shown as *branch style*, and the Ca^{2+} are marked as *green spheres*. **c**, **d** show the time dependence of the distance between Ca^{2+} and carbonyl oxygen atom

Fig. 4 Sequence alignment of Ca^{2+} bowl with the template and the secondary structure prediction of it. The residues are colored in similarity

binding site. Existing prediction approaches of Ca^{2+}-binding site may be classified into two general categories based on the types of input data they take: sequence or structural information [39, 40]. Pure sequence-based approaches are only applicable for those proteins which have specific consensus sequence, such as the EF hand or IQ motif in calmodulin. Meanwhile, prediction approaches based on structural information are only applicable for those who have integral crystal structure. However, many of the proteins have neither X-ray crystallographic structures nor typical Ca binding sequence motif among the whole superfamily of CaBPs. To the best of our knowledge, this is the first study which combines the FHM with MD simulation for sequence-structure-based site recognition in CaBPs. For experimentalists, the best way to identify the Ca^{2+} binding site is Ala scan, which will be a long and tortuous process. This study aimed at reducing the scope of the further experimental mutation. We first selected 18 crystal structures of CaBPs to confirm if MD simulation can identify the Ca^{2+}-binding sites, and then, we move to BK channel to confirm if FHM and MD can predict the Ca^{2+}-binding sites in RCK1 and RCK2.

It has been thought that CaBPs bind Ca^{2+} through weak electrostatic interactions. The calcium-protein complexes are highly charged systems. There are many local energy minimum points, the Ca^{2+}-binding site may be one of a global energy minimum in the CaBPs. For recognition of the site, we have considered ion-protein binding as the ligand-acceptor interaction. Because Ca^{2+} is geometrically centrosymmetric, the keys-locks based docking methods didn't come to any reasonable result from the conformational point of view. Then, we thought of the ion in protein system for molecular dynamics simulations. The right initial position of Ca^{2+} is important for correct simulation results. If we placed Ca^{2+} randomly in the protein-water system, this kind of simulation didn't give any methodical results. Finally, we address this problem by placing the ions into the global minimum of the electrostatic potential.

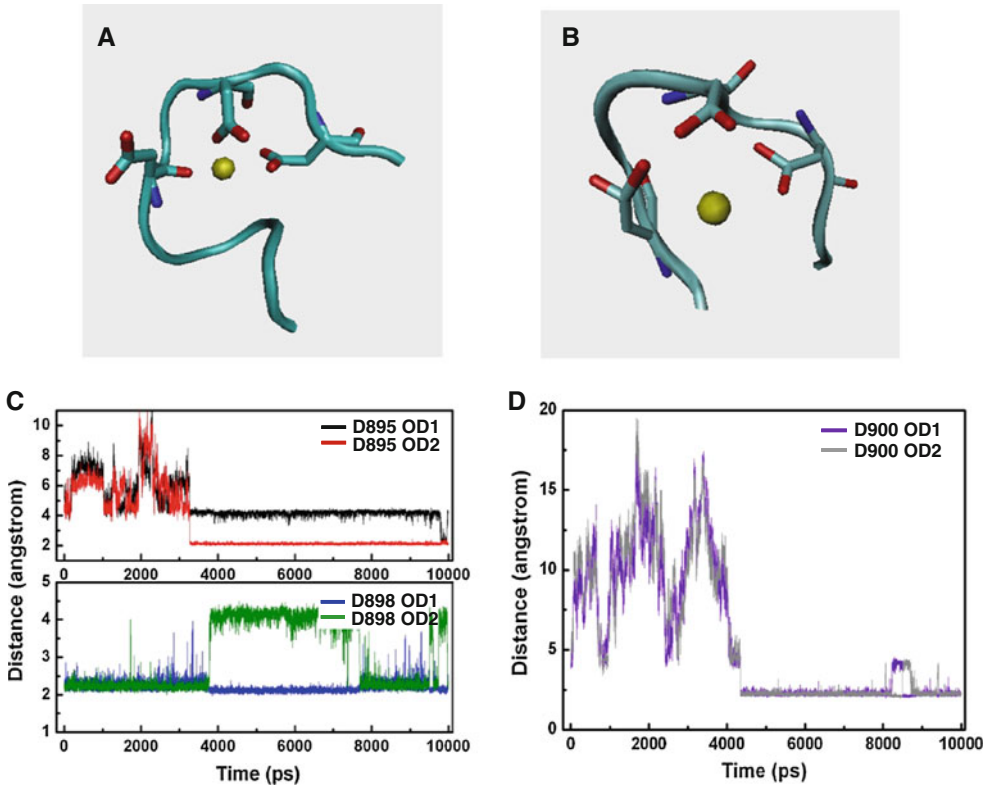

Fig. 5 FHM and MD simulation predicted the Ca^{2+}-binding residues in fragment 2 of BK channel. **a** Stereoview of the crystal structure of Ca^{2+} bowl. **b** FHM models the structure of fragment 2 with Ca^{2+} loaded by MD. Asp895, Asp898, Asp900 are shown as branch style, and the Ca^{2+} are marked as green spheres. **c**, **d** show the time dependence of the distance between Ca^{2+} and corresponding carbonyl oxygen atom

Following this principle, MD simulation produced reasonable result with certain accuracy.

Protein crystal structures are a potentially rich source of functional information. A thorough statistical analysis of known calcium-binding proteins deposited in Protein Data Bank gives reference values of various parameters characterizing geometric and chemical features in calcium-binding sites including distances, angles, coordination numbers and ligand types. In general, Ca^{2+} binding site is commonly consisted of 2–6 residues which provide 4-8 carbonyl oxygen atom, and all the ligand oxygen atoms form hexahedral structure to bind Ca^{2+} with the distance of 2–4 Å. Our data show that the simulated states meet the geometric characteristic of the binding site. Most of the simulated Ca^{2+}-O distances are less than 4 Å; A few distances are more than 4 Å (Table 1 and Supporting Tables). However, the crystal structure is a static conformation of the CaBPs, but Ca^{2+}-binding with CaBPs is a dynamical process and the side chains of the ligand residues are much flexible. It is hardly to say if the crystal structures show every physiological state of the ion-protein complex.

Combined with FHM, MD confirmed the two Ca^{2+}-binding sites in RCK1 and RCK2 domain of BK channel, respectively. For BK channel, the Ca^{2+}-free, closed, state of the gating ring is defined 2 years ago [20, 36], yet, we still don't know how the Ca^{2+} bind to the two SDM determined binding sites. This provides us a good choice to evaluate the method of FHM and MD simulation in detecting the binding site of CaBPs. When the simulation was almost done, Ca^{2+}-bound conformation of the gating ring of BK channel was presented [37], which provide a pretty reference. The models of the Ca^{2+} bowl constructed with FHM shows that the residues in Ca^{2+} bowl together with D895, D898 and D900 form a semicircular architecture and coordinate the Ca^{2+} in the center. The side chains of D895/D898/D900 coordinate Ca^{2+}.

Regarding the RCK1 domain, it has been reported that mutations of D362 or D367 weaken the apparent affinity of Ca^{2+} [19]. Because there is no Ca^{2+}-loaded crystal structure of RDK1 domain, the detailed binding mechanism is still unclear. Based on our data, besides D362 and D367, D369 also contributes to stabilize Ca^{2+}. D369 is reported to be important for the allosteric coupling between Ca^{2+} binding and channel activation. However, the D369G mutation, which is associated with human syndrome of generalized epilepsy and paroxysmal dyskinesia, enhanced the apparent affinity of Ca^{2+} through reducing the flexibility of the entire AC region [41]. Our hypothesis is that

Ca^{2+} binding at RCK1 will reduce the flexibility of the same region, i.e., D369G mutation mimics the Ca^{2+} binding effect on the channel gating. The additional MD simulation validate the idea that Ca^{2+} binding cause the entire AC region to move as a more rigid entity.

Conclusion

Homology modeling, combined with MD simulation, has been used successfully for many biological macromolecules. In this paper, the author developed a theoretical approach which combined the FHM and MD simulation, aimed at predicting the binding site and gaining a better understanding of the binding mechanism of the ions-protein complex. The result of theoretical approach in predicting Ca^{2+} binding site has been discussed; the effectiveness of the methodology is demonstrated.

In the absence of a crystal structure, the fragment homology model with further MD simulations of our study provides an alternatively and relatively feasible way to identify the relevant structural and dynamical information. We strongly believe that a combination of large-scale computational methods and experimental approaches will reveal many other interesting properties of the CaBPs family. We think results of the current study shed some light to the binding mode of CaBPs for further computational studies.

Acknowledgments The work is supported by National Natural Science Foundation of China Grants 11175055 to YZ, 11247010 to HA, and by Natural Science Foundation of Hebei Province grant C2012202079 to HA, A2011202129 to MJ.

References

1. Batistic O, Kudla J (2012) Analysis of calcium signaling pathways in plants. Biochim Biophys Acta 1820:1283–1293
2. Haiech J, Audran E, Feve M, Ranjeva R, Kilhoffer MC (2011) Revisiting intracellular calcium signaling semantics. Biochimie 93:2029–2037
3. Nedergaard M, Rodriguez JJ, Verkhratsky A (2010) Glial calcium and diseases of the nervous system. Cell Calcium 47: 140–149
4. Ramadan JW, Steiner SR, O'Neill CM, Nunemaker CS (2011) The central role of calcium in the effects of cytokines on beta-cell function: implications for type 1 and type 2 diabetes. Cell Calcium 50:481–490
5. Tam BK, Shin JH, Pfeiffer E, Matsudaira P, Mahadevan L (2009) Calcium regulation of an actin spring. Biophys J 97:1125–1129
6. Bazzazi H, Kargacin ME, Kargacin GJ (2003) Ca2 + regulation in the near-membrane microenvironment in smooth muscle cells. Biophys J 85:1754–1765
7. Shin DW, Pan Z, Bandyopadhyay A, Bhat MB, Kim DH et al (2002) Ca(2 +)-dependent interaction between FKBP12 and calcineurin regulates activity of the Ca(2 +) release channel in skeletal muscle. Biophys J 83:2539–2549
8. Perochon A, Aldon D, Galaud JP, Ranty B (2011) Calmodulin and calmodulin-like proteins in plant calcium signaling. Biochimie 93:2048–2053
9. Donato R (2001) S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 33:637–668
10. Erskine PT, Beaven GD, Hagan R, Findlow IS, Werner JM et al (2006) Structure of the neuronal protein calexcitin suggests a mode of interaction in signalling pathways of learning and memory. J Mol Biol 357:1536–1547
11. Lee US, Cui J (2010) BK channel activation: structural and functional insights. Trends Neurosci 33:415–423
12. Hartzell C, Putzier I, Arreola J (2005) Calcium-activated chloride channels. Annu Rev Physiol 67:719–758
13. Grabarek Z (2011) Insights into modulation of calcium signaling by magnesium in calmodulin, troponin C and related EF-hand proteins. Biochim Biophys Acta 1813:913–921
14. Grabarek Z (2005) Structure of a trapped intermediate of calmodulin: calcium regulation of EF-hand proteins from a new perspective. J Mol Biol 346:1351–1366
15. Nalefski EA, Falke JJ (1996) The C2 domain calcium-binding motif: structural and functional diversity. Protein Sci 5: 2375–2390
16. Pappa H, Murray-Rust J, Dekker LV, Parker PJ, McDonald NQ (1998) Crystal structure of the C2 domain from protein kinase C-delta. Structure 6:885–894
17. Bao L, Kaldany C, Holmstrand EC, Cox DH (2004) Mapping the BKCa channel's "Ca^{2+} bowl": side-chains essential for Ca^{2+} sensing. J Gen Physiol 123:475–489
18. Yang YD, Cho H, Koo JY, Tak MH, Cho Y et al (2008) TMEM16A confers receptor-activated calcium-dependent chloride conductance. Nature 455:1210–1215
19. Xia XM, Zeng X, Lingle CJ (2002) Multiple regulatory sites in large-conductance calcium-activated potassium channels. Nature 418:880–884
20. Yuan P, Leonetti MD, Pico AR, Hsiung Y, MacKinnon R (2010) Structure of the human BK channel Ca2 + -activation apparatus at 3.0 A resolution. Science 329:182–186
21. Roberts E, Eargle J, Wright D, Luthey-Schulten Z (2006) MultiSeq: unifying sequence and structure data for evolutionary analysis. BMC Bioinformatics 7:382
22. Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, et al. (2006) Comparative protein structure modeling using Modeller. Curr Protoc Bioinformatics Chapter 5: Unit 5 6
23. Laskowski RA, Macarthu MW, Moss DS, Thornton JM (1993) PROCHECK: a program to check the stereochemical quality of protein structures. J App Cryst 26:283–291
24. Bashford D (1997) An object-oriented programming suite for electrostatic effects in biological molecules An experience report on the MEAD project. Sci Comput Object-Oriented Parallel Environ Lecture Notes Comput Sci 1343:233–240
25. Bashford D, Gerwert K (1992) Electrostatic calculations of the pKa values of ionizable groups in bacteriorhodopsin. J Mol Biol 224(2):473–486
26. Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E et al (2005) Scalable molecular dynamics with NAMD. J Comput Chem 26:1781–1802
27. MacKerell AD, Bashford D, Bellott, Dunbrack RL (1998) All-atom empirical potential for molecular modeling and dynamics studies of proteins. J Phys Chem B 102:3586–3616
28. Kal'e Laxmikant, Skeel R, Bhandarkar M, Brunner R, Gursoy A et al (1999) NAMD2: greater scalability for parallel molecular dynamics. J Comput Phys 151:283–312

29. Procyshyn RM, Reid RE (1994) A structure/activity study of calcium affinity and selectivity using a synthetic peptide model of the helix-loop-helix calcium-binding motif. J Biol Chem 269:1641–1647
30. Chattopadhyaya R, Meador WE, Means AR, Quiocho FA (1992) Calmodulin structure refined at 1.7 A resolution. J Mol Biol 228:1177–1192
31. Chou JJ, Li S, Klee CB, Bax A (2001) Solution structure of Ca(2 +)-calmodulin reveals flexible hand-like properties of its domains. Nat Struct Biol 8:990–997
32. Cui J, Yang H, Lee US (2009) Molecular mechanisms of BK channel activation. Cell Mol Life Sci 66:852–875
33. Zeng XH, Xia XM, Lingle CJ (2005) Divalent cation sensitivity of BK channel activation supports the existence of three distinct binding sites. J Gen Physiol 125:273–286
34. Kim HJ, Lim HH, Rho SH, Bao L, Lee JH et al (2008) Modulation of the conductance-voltage relationship of the BK Ca channel by mutations at the putative flexible interface between two RCK domains. Biophys J 94:446–456
35. Kranjc A, Anselm C, Carloni P, Blaney FE (2007) Structural models of human big conductance calcium- and voltage-gated potassium channels. Comput Phys Commun 177:21–26
36. Wu Y, Yang Y, Ye S, Jiang Y (2010) Structure of the gating ring from the human large-conductance Ca(2 +)-gated K(+) channel. Nature 466:393–397
37. Yuan P, Leonetti MD, Hsiung Y, MacKinnon R (2012) Open structure of the Ca2 + gating ring in the high-conductance Ca2 + -activated K + channel. Nature 481:94–97
38. Bhattacharya A, Padhan N, Jain R, Bhattacharya S (2006) Calcium-binding proteins of Entamoeba histolytica. Arch Med Res 37:221–225
39. Liu T, Altman RB (2009) Prediction of calcium-binding sites by combining loop-modeling with machine learning. BMC Struct Biol 9:72
40. Wang X, Zhao K, Kirberger M, Wong H, Chen G et al (2010) Analysis and prediction of calcium-binding pockets from apo-protein structures exhibiting calcium-induced localized conformational changes. Protein Sci 19:1180–1190
41. Yang J, Krishnamoorthy G, Saxena A, Zhang G, Shi J et al (2010) An Epilepsy/Dyskinesia-Associated Mutation Enhances BK Channel Activation by Potentiating Ca2 + Sensing. Neuron 66:871–883