CHAPTER-III

Modeling of EhCaBP5-IQ motif peptide Complex

4.1 Abstract

The surface plasmon resonance (SPR) assay, cell co-localization and pull down assay confirm our hypothesis and EhCaBP5 was found to interacting with Myosin IB *in vitro* as well as *in vivo*. To know how these two proteins may interact with each other, we modeled EhCaBP5 in complex with IQ-motif peptide. The various complex structure of CaM-target protein and structures of myosin in complex with its light chain provided basis for the modeling of EhCaBP5-IQ motif complex. The EhCaBP5-IQ motif complex model was obtained by using Rosetta FlexPepDock web server (London et al., 2011). The EhCaBP5-IQ motif complex model adopts more open C-terminal conformation and the open C-terminal lobe accommodates IQ-motif peptide in the cleft. N- and C-terminal lobes of EhCaBP5 move apart and wrap up the peptide. The interaction of EhCaBP5 with IQ motif is mainly governed by hydrophobic residues and nonbonded interaction of complex model. Superimposition of native EhCaBP5 structure on EhCaBP5-IQ motif complex model helped to identify structural changes in EhCaBP5 needed to form a complex with IQ motif. However, the peptide binding did not lead to global changes, as binding of peptide to EhCaBP5 did not interfere or alter the structure of N-terminal domain of EhCaBP5 and C-terminal half adopt extended conformation. Orientation of the C-terminal part of EhCaBP5 changed as it moved 15 degree as compared to the native EhCaBP5 structure, to accommodate the peptide resulting in stretching out of loop connecting the two domains. To understand local conformational changes at C-terminal upon peptide binding we tried to superimpose the C-terminal of the model upon the crystal structure of EhCaBP5. The r.m.s.d. of the alignment was 0.601 suggesting movement of the C-terminal as compared to the N-terminal. The overall length of native EhCaBP5 from head to tail is 47.7 Å, whereas length of EhCaBP5-IQ motif complex is 55.1 Å, clearly indicating elongated conformation adopted by EhCaBP5 after peptide binding.

4.2 Introduction

Calmodulin (CaM) is a ubiquitous, calcium-binding protein that can bind to and regulate function of variety of protein targets, thereby affecting many different cellular functions. CaM is a small, dumb bell-shaped protein that is a structural homologue of the calcium binding subunit of troponin (TnC), but rather than having a single known regulatory function, CaM binds to a diverse array of target proteins to modulate their functions in response to calcium signals (Yanling et al., 2012, Chin et al., 2000, Vetter et al., 2003, Crivici et al., 1995). Structurally CaM is evolved in such a manner so that it can interact with broad range of target peptides in the calcium bound as well as in apo state. CaM can bind with high affinity to a relatively small α-helical region of many proteins. High degree of sequence identity has been observed in CaM amino acid residues among vertebrates, with multiple genes encoding identical CaM's (Friedberg F., 1990). This high degree of conservation may be essential for the maintenance of interaction with a diverse family of CaM binding proteins (Crivici et al., 1995). Various Structures of Ca^{+2} bound CaM with target peptide are reported (Andriyka et al., 2002, Meador et al., 1992, Marius et al., 1993, Shen et al., 2002, Rellos et al., 2010, Köster et al., 2011,). Apart from these Ca^{+2} bound CaM and target peptide structure, there are also evidences of Ca^{+2} free CaM binding to and regulating a variety of proteins function whose target sequence are far more restricted (Houdusse et al., 1996, 2006). The CaM interacts to myosin/target protein in calcium dependent or independent manner. Based on the variety of CaM-target complex structure mainly four recognition motifs are discussed.

These motifs are (1) Calcium-independent CaM binding motifs, (2) Calcium-dependent CaM binding motifs, (3) The 1-8-14 CaM binding motif (4) The 1-5-10 CaM binding motif. Apart from these conserved motifs. Some additional CaM binding motifs are also present and these motifs are likely to present in CaM dependent proteins (Rhoads et al., 1997). CaM/CaM-like protein have tendency to interact with myosin and this interaction are linked with various cellular signalling process. Myosin constitute a diverse superfamily of actin-based mechanoenzymes that are involved in many essential cellular process including cytokinesis, contractile vacuole function, secretion, phagocytosis, pseudopod formation, vesicular trafficking and polarized cell growth (Houdusse et al., 2006). All myosin contains three functional domains a globular N-terminal head domain that contain actin and ATP binding site. Head domain is followed with alpha helical neck domain (lever arm) and it is followed by tail domain. The lever arm contains at least one IQ motif, the consensus

sequence for the IQ motif is IQxxxRGxxxR (where X is any amino acid), and these motifs are stabilized by the binding of CaM (Houdusse et al., 1999, Houdusse et al., 2006). The mode of CaM-target protein binding was first time revealed by the crystal structure of regulatory domain of scallop myosin at 2.8 Å resolution. This structure provided the first description of the physical basis for the binding of both the regulatory (RLC) and essential (ELC) LCs to the two IQ motifs in the HC of this myosin (Xie et al., 1994). The model represent that lobes of the ELC and RLC adopt three distinct conformations when bound to the HC (Houdusse et al., 1995). Two of these conformations have been identified previously in CaM (Babu et al., 1985) and troponin C (Herzberg et al., 1985) and corresponds to the conventional "open" and "closed" forms that a lobe assumes when divalent cations are bound or absent, respectively. The third state is an unusual "semi-open" conformation in which no metal is bound and is found in the C-terminal lobes of each of the two LCs (Houdusse et al., 1995). On the basis of above described complex formation mode CaM-target binding model have been proposed (Figure 1).

This model was validated with the help of various CaM-target proteins complex models, like Mlc1p is a calmodulin-like myosin light chain that binds to IQ motifs of a class V myosin, Myo2p, and an IQGAP related protein, Iqg1p, play a role in polarized growth and cytokinesis in *Saccharomyces cerevisiae*. It has been shown that the crystal structures of Mlc1p bound to IQ2 and IQ4 of Myo2p adopts two different conformations. Mlc1p when bound to IQ2, adopts a compact conformation in which both the N- and C-lobes interact with the IQ motif whereas, in the complex with IQ4, the N-lobe no longer interacts with the IQ motif, resulting in an extended conformation of Mlc1p, and it was expected that the extended light chain conformation could play a role in myosin localization or binding to targets and effectors (Terrek et al., 2003, 2005). Crystal structure of calcium-free calmodulin bound to the first two IQ motifs of the murine myosin V heavy chain reveals an unusual calmodulin conformation. The C-terminal lobe of each calmodulin adopts a semi-open conformation that grips the first part of the IQ motif (IQxxxR), whereas the N-terminal lobe adopts a closed conformation that interacts more weakly with the second part of the motif (GxxxR) (Houdusse et al., 2005).

The crystal structures of squid myosin (Yang et al., 2007) provided basis for the modeling of EhCaBP5-IQ motif of Myosin IB. The complex model provides the insight of probable interaction between EhCaBP5-IQ motif complex.

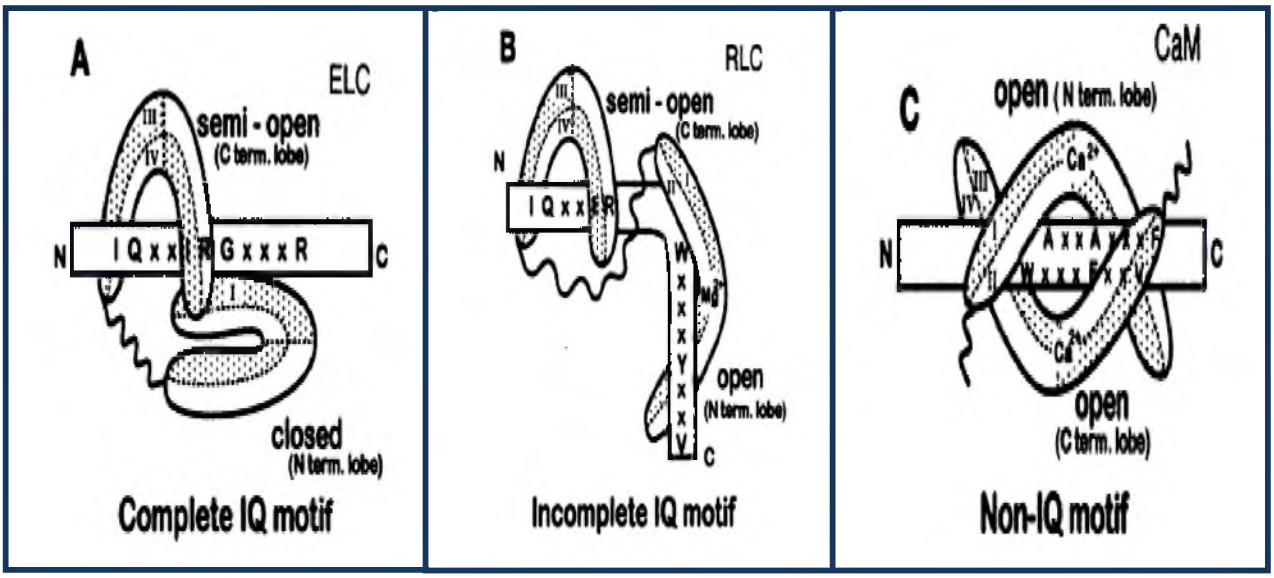

Figure 1: Schematic drawing of modes of calmodulin target binding: Each half lobe corresponds to a pair of helices (e.g., A/D, B/C, F/G, E/H). The domains to which they belong are noted in the figure to indicate the polarity of binding. (Domains III and I are stippled.) Note that in the ELC, N-terminal lobe domain I makes only surface interactions with the target peptide. For clarity, the complete inter lobe linker is not shown in CaM (C).

4.3 Materials and Methods

4.3.1 Modeling and docking of EhCaBP5 with IQ motif

The initial or the starting structure of EhCaBP5 in complex with the desired IQ motif was obtained using flexible superimposition (Mosca et al., 2008) of the crystal structure of EhCaBP5 on crystal structure of ELC from squid myosin (Yang et al., 2007). The course grained complex model of CaBP5-IQ motif was then used for the molecular docking simulations with IQ motif peptide using Rosetta FlexPepDock web server (London et al., 2003). The Rosetta FlexPepDock protocol optimizes the protein–peptide complex using Monte-Carlo algorithm along with energy minimization. In this study we have used 200 models for refinement and chose the best model based on their Rosetta generic full-atom energy score. The images were prepared using Pymol (DeLano WL (2002) visualization software.

4.4 Result

4.4.1 Model of EhCaBP5 and IQ motif complex

The molecular details of EhCaBP5-Myosin IB IQ motif interaction were studied using molecular docking simulations. As crystal structure and EF-hand motif conformation of EhCaBP5 resembled ELC of myosin, the coarse grained model was obtained using the crystal structure of squid myosin ELC (Yang et al., 2007). The peptide bound conformation of EhCaBP5 was obtained by employing flexible superimposition protocol of RAPIDO structural alignment software (Mosca et al., 2008). The superimposition of EhCaBP5 on ELC of squid myosin structure yielded r.m.s.d. of 0.96 Å. The peptide (IQ motif) was modelled using the crystal structure of squid myosin (Yang et al., 2007). To eliminate bad contacts within the peptide bound complex of EhCaBP5-IQ motif, the model was further refined using AMBER12 package (Hornak et al., 2006) for energy minimization. The minimized complex was then used for molecular docking simulations. The best model obtained after docking was used for further analysis (Figure 2A, 2B).

The peptide bound EhCaBP5 model adopts more open C-terminal conformation and open C-terminal lobe accommodates IQ-motif peptide in the cleft. N- and C-terminal lobes of EhCaBP5 move apart and wrap up the peptide. The N-terminal of peptide interacts with C-terminal lobe of EhCaBP5 and C-terminal of peptide binds to N-terminal domain of EhCaBP5 molecule (Figure 2A). The interaction of C-terminal peptide to N-terminal EhCaBP5 is through the residues F-15, G-17, E-27, S-30, R-33 and M-39, the N-terminal region of EhCaBP5 comes in contact with C-terminal of K-789, K-792, G-793, N-796, R796 and S-800 (Figure 2A). The majority of residues from C-terminal domain of EhCaBP5 interact with N-terminal region of peptide (Figure 2A), in this interaction C-terminal lobe opens up to accommodate the peptide. The protein forms three hydrogen bonds, between the backbone of Phe-15 with side chain of Ser-800 and the side chains Ser-30 and Asn-109 with Gly-793 and Lys-792 of the peptide.

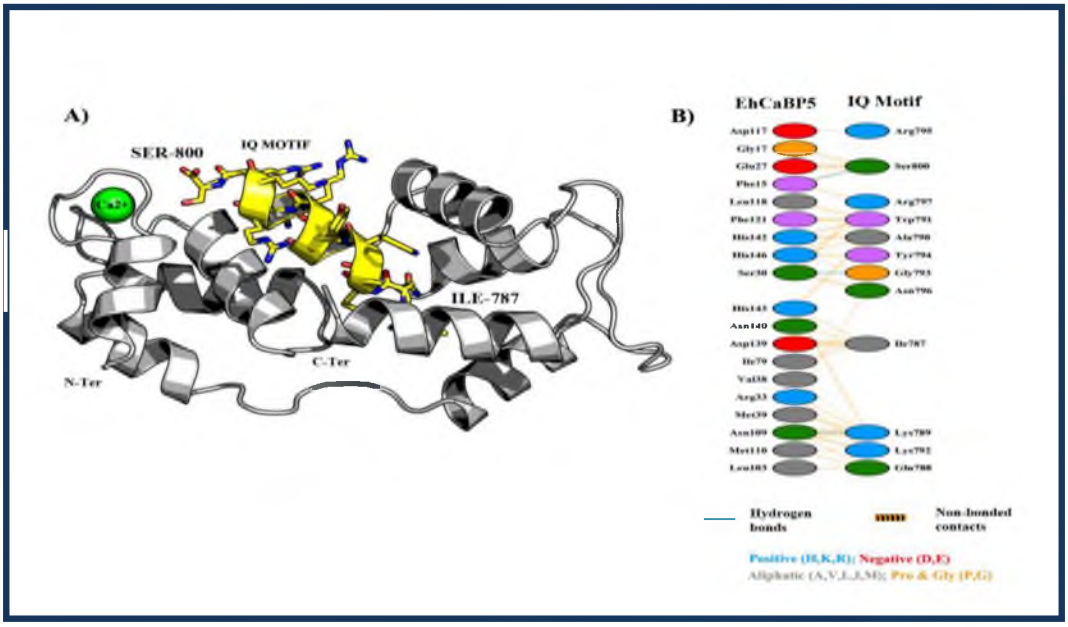

Figure 2 A & B: Protein-peptide complex model and interaction: A) Rosetta Docked low energy model of EhCaBP5 and IQ motif complex. The IQ motif is shown in Stick represented. B) Schematic representation of contact between EhCaBP5 and peptide. The interaction shown by dotted orange lines are the non-bonded interactions, in which the width of the striped line is proportional to the number of atomic contacts. The Blue line between any two residues indicates the number of potential hydrogen bonds between them.

4.4.2 Comparison of Apo and complex structure of EhCaBP5

Superimposition of native EhCaBP5 structure on EhCaBP5-IQ motif complex model helped to identify structural changes in EhCaBP5 needed to form a complex (Figure 3) with IQ motif. However, the peptide binding did not lead to global changes, as binding of the peptide did not interfere or alter the structure of N-terminal domain of EhCaBP5. N-terminal of the crystal structure aligned nicely on the N-terminal of the Rosetta docked model with an r.m.s.d. of 0.072 (residue number 8 to 65). Orientation of the C-terminal part of EhCaBP5 changed as it moved 15 degrees as compared to the native EhCaBP5 structure, to accommodate the peptide resulting in stretching out of loop connecting the two domains (Figure 3). To understand local conformational changes at C-terminal upon peptide binding we tried to superimpose the C-terminal of the model upon the crystal structure of EhCaBP5. The r.m.s.d. of the alignment was 0.601 suggesting movement of the C-terminal as compared to the N-terminal. The orientation of the C-terminal loops determines altered direction of the helices that helps the molecule to have an open conformation needed to bind the peptide. The overall length of native EhCaBP5 from head to tail is 47.7 Å, whereas length of EhCaBP5-IQ motif complex is 55.1 Å, clearly indicating elongated conformation adopted by EhCaBP5 after peptide binding.

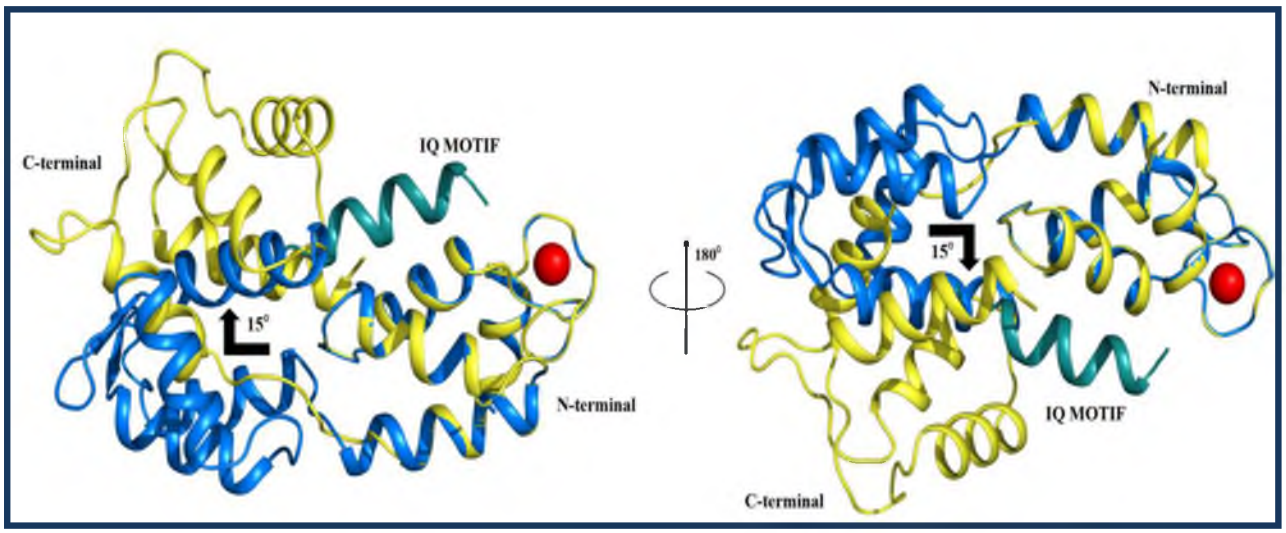

Figure 3: Superimposition of native EhCaBP5 to peptide bound complex model of EhCaBP5: Superimposition of both models clearly indicates that peptide bound model goes under structural modification as it moves 15° away from the native structure, only C-terminal half moves to accommodate peptide and N-terminal half remains is native position.

4.5 Discussion

The model of calcium bound protein-peptide complex provided structural evidence on how myosin IB may interact to EhCaBP5. Maximum participation for the interaction between protein-peptide complexes is coming from the C-terminal globe of EhCaBP5 whereas participation from N-terminal part is very low for the interaction. Due to the low participation for the interaction of N-terminal part of EhCaBP5, the N-terminal half structure remains unchanged after peptide binding. The C-terminal part of EhCaBP5 actively participates in interaction with peptide. C-terminal globe open up to create a cleft or pocket for the binding of peptide. Modeling study suggests that EhCaBP5 adopt extended conformation when it interacts to myosin. It has been also shown in the crystal structures of calmodulin like light chain of Mlc1p bound to IQ4 peptide of Myo2p adopt extended conformation and it was expected that the extended conformation could mediate the formation of ternary complexes during protein localization and/or partner recruitment (Terrek et al., 2003). Therefore we expect that the extended conformation of EhCaBP5 with IQ motif may allow to interact with other molecules/partner during the various cellular processes.