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The On-off Switch in Regulated Myosins: Different Triggers but Related Mechanisms

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Abstract

In regulated myosins, motor and enzymatic activity are toggled between on- and off-states by a switch located on its lever arm, or regulatory domain (RD). This region consists of a long alpha-helical "heavy chain" stabilized by a "regulatory" and an "essential" light chain. The on-state is activated by phosphorylation of the regulatory light chain of smooth muscle RD, or by direct binding of Ca²⁺ to the essential light chain of molluscan RD. Crystal structures are available only for the molluscan RD. To understand the pathway between the on and off states in more detail, we have now also determined the crystal structure of a molluscan (scallop) RD in the absence of Ca²⁺. Our results indicate that loss of Ca²⁺ abolishes most of the interactions between the light chains and may increase flexibility of the RD heavy chain. We propose that disruption of critical links with the C-lobe of the regulatory light chain is the key event initiating the off-state in both smooth muscle and molluscan myosins.

Keywords

Regulated myosins; smooth and molluscan muscle; X-ray crystallographic structure; scallop regulatory domain/lever arm; Off-state initiation

Introduction

The muscle machinery consists of thick filaments of myosin that translocate along thin filaments of actin (F-actin) powered by ATP hydrolysis. The motor that drives this action is located in the myosin head, which consists of a motor domain and a lever arm domain, here called the regulatory domain (RD). The RD comprises the long heavy chain (HC) α -helix of the lever arm, stabilized by two types of light chain, the essential light chain (ELC) and the regulatory light chain (RLC)1. Each of the light chains, which belong to the calmodulin

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Abbreviations: CaM, ELC, EM, HC, HMM, MLCK, RD, RLC

Accession Numbers

Coordinates have been deposited with the Protein Data Bank with accession no. **XXXX** for Ca²⁺-RD and 3JTD for D19A-RD.

(CaM) superfamily of proteins², consists of an N-lobe (helices A, B, C, and D) and a C-lobe (helices E, F, G, and H) that have specific interactions with the HC³; ⁴. A flexible linker joins helices D and E. The light chains act as regulatory switches in molluscan (e.g., scallop) and vertebrate smooth muscle myosins but not in the myosins of vertebrate striated muscles. In all muscles, contraction is initiated by the release of Ca^{2+} stored in vesicles. In vertebrate striated muscles, contraction is initiated by the binding of Ca^{2+} to a troponin-tropomyosin complex on F-actin. Activity of isolated myosin, however, remains locked in an on-state regardless of Ca^{2+} concentration (for reviews, see refs.⁵; ⁶). In regulated myosins, the light chains control myosin activity even in the absence of actin⁷.

The Ca^{2+} -mediated triggers of molluscan and smooth muscle myosins are different. In molluscan muscle myosin, motor and enzymatic activity are switched "on" when Ca^{2+} binds directly to the ELC (Figure 1)⁷; ⁸; ⁹. The ELC of molluscan myosins contains a unique sequence of five residues that provide ligands for the binding of Ca^{2+} even in the presence of excess magnesium. The Ca^{2+} -chelating ability of these residues is stabilized by interactions with a crucial glycine residue of the RLC (Gly117 in scallop)³; ⁴; ⁹. The highly homologous vertebrate smooth muscle myosin lacks Ca^{2+} binding ligands¹⁰, and force generation is activated indirectly when Ca^{2+} is bound by calmodulin, initiating a series of events that include activation of myosin-light chain kinase (MLCK) and finally phosphorylation of the RLC (for review, see ref. 11). Each of these biochemical steps presents a potential entry point for regulatory control of smooth muscle contraction and its rate of activation. As a result, the relatively slow response of smooth muscle to Ca^{2+} release allows a variety of controls over regulation important for the survival of the organism. The RLC of vertebrate smooth muscle myosin has an extension on the N-terminus that provides a binding site for MLCK, including an arginine critical for kinase activity¹². By contrast, the scallop RLC has a shorter extension and lacks this arginine (it is replaced by lysine). As a result, the scallop RLC cannot be phosphorylated by MLCK. Thus, although both light chains are necessary for maintaining the RD structure, regulation of smooth muscle myosin depends only on the RLC, whereas regulation of molluscan myosin depends on both the RLC and ELC. In short, the initial step in the regulatory switch mechanism (i.e. the trigger) of molluscan myosin is clearly different from that of smooth muscle¹³.

There are, however, similarities between regulation in molluscan and smooth muscle myosins (see, for example, ref. 14). There is evidence that in both myosins one or more of the last several carboxy-terminal residues of the RLC are required for regulation¹⁵; ¹⁶. Of these residues, scallop Lys149 (gizzard smooth Lys163), is conserved in all regulated myosins but is absent from all unregulated myosin II's². In both isoforms, regulation requires a myosin dimer containing two myosin heads and a portion of the coiled-coil rod which connects them¹⁴; ¹⁷. Heavy meromyosin (HMM) is an example of a regulated two-headed myosin fragment. In a hybrid of HMM containing scallop heavy chains, scallop ELCs, and smooth muscle RLCs, the MgATPase and motor activity can be regulated by either phosphorylation or by Ca^{2+} -binding¹⁸; ¹⁹. This finding indicates that the smooth muscle RLC contains all the components required by both trigger mechanisms, providing that the appropriate ELC is present. If we assume that both triggers can induce the same off-state conformation, then the hybrid results suggest that both phosphorylation (the smooth muscle trigger) and Ca^{2+} -binding (the molluscan trigger) are two separate pathways activating the same underlying regulatory machinery. Cryo-EM studies indicate that the asymmetric off-state conformation of smooth muscle (involving quaternary interactions between two myosin heads)²⁰; ²¹; ²² closely resembles that of scallop²³, as well as tarantula myosin thick filaments (which are regulated by phosphorylation as in smooth muscle myosin)²⁴. In summary, although the initial trigger for regulation differs in scallop and smooth muscle myosins, experimental results suggest that the off-state conformation -

and at least some components of the regulatory machinery - may be similar for both types of myosin.

We do not yet know how dissociation of Ca^{2+} from scallop RD sets in motion a conformational change leading to the off-state. In scallop myosin, regulation is abolished if the last eleven carboxy-terminal residues of its RLC are substituted with different amino acids¹⁶, but specific binding of Ca^{2+} remains intact. This finding demonstrates that the RLC has distinct functional regions involved in switch activation. This view is also supported by a D39A point mutation in scallop RLC, which weakens RLC binding and results in loss of regulation without loss of Ca^{2+} binding¹⁶.

In order to investigate Ca^{2+} -mediated regulation of scallop myosin, 2.8 Å and 2.0 Å crystal structures of scallop Ca^{2+} -bound RD have been determined in this laboratory and were interpreted as corresponding to the switch as it appears in the on-state^{3; 4}. It was proposed that the absence of Ca^{2+} from the its ELC binding site might result in electrostatic repulsion between the coordinating oxygen atoms, leading to a local conformational change that would weaken ELC/RLC interactions and allow more flexibility of the heavy chain. This flexibility would allow formation of off-state interactions between two myosin heads that would affect ATPase activity and contractility³. For example, head-head contacts between the motor domains could trap them in conformations that prevent catalysis and mechanical motion, such as indicated by the cryo-EM studies cited above^{20; 21; 22; 24}. A structure of Ca^{2+} -free scallop myosin has not yet been reported, however, to confirm this hypothesis. Moreover, as a result of proteolysis, a number of N-terminal residues of the RLC were missing from previously published scallop RD crystal structures, including a serine (Ser6) at a position corresponding to the regulatory phosphorylation site in homologous smooth muscle RLC. To date, the smooth muscle RD has not been crystallized. To extend the investigation of how the molluscan regulatory switch toggles myosin between the on- and off-states and in an attempt to gain some insight into the phosphorylation machinery of smooth muscle myosin, we have determined two scallop RD crystal structures, reconstituted with complete light chains. One is a 2.6 Å resolution structure of an RD containing an ELC D19A point mutation (D19A-RD), which prevents Ca^{2+} from binding to the regulatory trigger site (see below under "**The Ca^{2+} -RD Binding Site**" for preliminary work involving wild-type RD in the presence of EGTA). The other structure is of Ca^{2+} -RD at 2.1 Å resolution, to be used as a control for the conformation of the RLC N-terminus as well as the Ca^{2+} binding site. Our results indicate a weakening of ELC/RLC interactions as a result of local conformational changes at the Ca^{2+} -binding site and lead us to a simple hypothesis that accounts for the on-off switch in regulated myosins.

Results

Overall Conformation

The current structures reveal the first comparison between Ca^{2+} -bound and Ca^{2+} -free scallop RD. The overall conformation of the Ca^{2+} -free D19A-RD structure is similar to that observed in both the current and previous scallop Ca^{2+} -RD and Ca^{2+} -S1 crystal structures (Figure 1a)^{3; 4}. By contrast to the previous crystallographic analyses of partially proteolyzed RD, however, the current experiments include the entire N-terminus of the RLC. The crystal structures show that the N-terminus of the RLC adopts extended, partially disordered, conformations which are different in the Ca^{2+} -bound and Ca^{2+} -free structures. It is difficult, however, to relate these differences simply to the state of the Ca^{2+} binding site. Although the overall conformations of the Ca^{2+} -RD and Ca^{2+} -free D19A-RD structures are similar, we observe a local change at the Ca^{2+} binding site (Figure 1a,c), which appears to weaken electrostatic interactions between the two light chains and suggests greater flexibility when Ca^{2+} is not bound.

The Ca²⁺-RD Binding Site

In the Ca²⁺-RD structure, the Ca²⁺ ion is coordinated by seven oxygen ligands, in agreement with previous scallop RD crystal structures^{3; 4}. These ligands include three main-chain carbonyls (ELC residues Asp19, Gly23, and Ala27), three side-chain carboxyl oxygen atoms (residues Asp19, Asp22, and Asp25), as well as one water molecule (Figure 1b). In the absence of Ca²⁺ (Figure 1c), there is no electron density for the water ligand. In addition, the Asp22 side-chain is turned away from the Ca²⁺ binding site. Unlike the Ca²⁺-RD structure, the electron density for ELC residue Gly23 in D19A-RD is difficult to interpret, but the carbonyl group clearly does not interact with the Ca²⁺ site and appears to have rotated away from it. Neighboring residue Arg24 is partially disordered. The side-chain of residue Asp25 now occupies two alternative conformations, one pointing toward the Ca²⁺ site and one pointing away from it. In preliminary studies, we crystallized wild-type RD in the presence of EGTA to remove Ca²⁺. Electron density could still be seen, however, at the Ca²⁺-binding site (results not shown), and we could not determine whether that density was the result of Ca²⁺ or water. The D19A-RD construct was designed to ensure that no cation is bound at this site (see Materials and Methods). Difference Fourier ($F_o - F_c$) electron density is still visible in the Ca²⁺ binding site of D19A-RD, although there are too few coordinating oxygen ligands to justify a Ca²⁺ atom at that position. An atom at that location can readily form hydrogen bonds with the carboxyl groups of Asp19 and Ala27. This $F_o - F_c$ density vanishes if a water molecule is modeled into the site. Modeling Mg²⁺ or Ca²⁺, however, results in a negative density. Thus, the site is probably occupied by a structural water, not a cation. This finding agrees with the observation that D19A-RD has a pK_{ca} of about 4, compared to a value of about 6 for other scallop myosin S1 and RD constructs^{25; 26}.

Compared to the Ca²⁺-RD coordinates, the D19A-RD coordinates suggest that removing the Ca²⁺ ion may increase mobility and weaken interactions at the ELC/RLC interface. The main-chain B-factors of ELC residues Asp22 to Gly26, comprising most of the Ca²⁺ binding loop, rise sharply compared to nearby residues (Figure 2) and spike at Arg24, an indication of high mobility. No such B-factor rise is seen in the equivalent residues of the Ca²⁺-RD structure.

Fewer ELC/RLC/HC Interactions

Critical electrostatic interactions between the two light chains have been lessened in the Ca²⁺-free structure, particularly in the supporting "meshwork" near Gly23 and Asp19 which is vital for coordination of the Ca²⁺ ion^{3; 4} (Figure 3b,c). The main-chain hydrogen bond between ELC residue Arg24 and RLC residue Gly117 has broken, as have two hydrogen bonds between the side-chains of ELC residue Arg24 and RLC residue Asp118. Some interactions between the RLC and HC by the ELC/RLC interface have become stretched to distances of 3.1 Å or greater, and a hydrogen bond between RLC residue Leu113 and HC residue Gln812 has been broken (Figure 3b,c). The overall interactions between this ELC loop and the HC have remained intact, however, including a 3.5 Å hydrophobic contact between the Phe20 benzene ring and the side-chain of HC residue Arg805. Adjacent HC residues, Lys796 through Gln804, have no interactions with either light chain, suggesting that this region may become more flexible than surrounding regions of the HC in the absence of strong interactions between the ELC, RLC, and HC, as previously proposed³. Indeed, these residues have elevated B-factors only in the D19A-RD structure, but not in the Ca²⁺-RD coordinates (Figure 2). This region is, in fact, precisely the location of a slight bend in the HC that brings the light chains close enough to interact. It is possible that the full range of HC flexibility cannot be visualized in the D19A-RD structure because of the stabilizing effects of crystal contacts, which are very similar to those of the Ca²⁺-RD structure. To summarize, in the Ca²⁺-free structure, interactions between the ELC, RLC, and

HC are weak near the ion's binding site, and, as a result, the adjacent solvent-exposed region of the HC is likely to be significantly more flexible than in Ca^{2+} -bound structures.

In comparing the current scallop RD structures, we find that the heavy chain is slightly more bent in Ca^{2+} -RD than in the Ca^{2+} -free D19A-RD, but it is not clear whether this is a result of the Ca^{2+} occupancy at its ELC binding site or a sampling of the normal plasticity of the RD HC. We find, however, that if we allow a modest straightening of the RD heavy chain by the ELC/RLC interface, we can improve the molecular fit to the recent HMM off-state cryo-EM map^{20, 21} (Himmel, unpublished result). Crystallographic experiments are in progress with a molluscan HMM off-state conformation to confirm this observation.

Discussion

One outstanding question is why the RLC carboxy-terminal residues of helix H are required for regulation in both molluscan and smooth muscle myosins, although those residues are not required for either Ca^{2+} or phosphate binding. As mentioned above, one of these residues, scallop Lys149 (gizzard smooth Lys163), is conserved only in regulated myosins². In the Ca^{2+} -RD structure, Lys149 forms a hydrogen bond with the side chain of RLC residue Thr83 (located on helix E), whereas in D19A-RD this hydrogen bond is broken. This observation led us to consider whether all regulated myosins can modulate the rigidity of the lever arm heavy chain by maintaining links only in the on-state between (1) the RLC N- and C-lobes and (2) between the RLC C-lobe and the ELC. This possibility would be consistent with the finding from a cross-linking study that the RLC N-lobe must rotate away from the ELC to achieve the off-state in regulated myosin²⁷. It would appear that a true on-state in smooth muscle myosin, and possibly even in molluscan myosin, might involve salt bridge and hydrogen bond contacts between RLC helices A, E, and H that would be further stabilized by a phosphate binding site near helix A. Breaking of these links - either indirectly by increasing mobility of the RLC C-lobe and ELC N-lobe (in molluscan muscle), or directly by dephosphorylation (in smooth muscle) - would permit myosin to fold into an off-state conformation. The helix A N-terminal residues that would be expected to interact with helix E are in close proximity to scallop RLC residue Ser6, whose equivalent in smooth muscle serves as the phosphorylation switch (Figure 4). Indeed, examination of the structure of the lever arm of chicken skeletal myosin²⁸ has previously led Trybus et al. to propose that phosphate might itself form one or more salt bridges between RLC helices A and H during the on-state of smooth muscle myosin²⁹. With the exception of the Lys149-Thr83 salt bridge, however, we do not consistently see links between helices A, E, and H in molluscan myosin structures to support these models. A structure of smooth muscle RD with a phosphorylated RLC would therefore be an important contribution to our understanding of myosin regulation. An important step, nevertheless, can be taken toward understanding the regulatory role of RLC helix H by noting that disruption of the Lys149-Thr83 salt bridge increases the degrees of freedom of movement between the RLC C- and N-lobes.

This notion allows us to posit a simple picture for the switch mechanism, derived directly from the crystallographic observation that Ca^{2+} dissociation weakens links with the RLC C-lobe in molluscan myosin. It is possible that the key to initiating an off-state is destabilization of the RLC C-lobe. In scallop myosin, this step would be achieved by breaking Ca^{2+} -dependent links to the ELC. The N-terminal segment of the RLC, which contains the phosphate binding site in smooth muscle myosin, is adjacent to the C-lobe of the RLC, including helices E and H (Figure 4). Although a smooth muscle RD structure has not yet been reported, it might well resemble that of squid S1³⁰ and chicken skeletal S1²⁸. In these structures, the RLC N-lobe is rotated in such a way that links between helices A, E, and H may be possible if the RLC N-terminus is phosphorylated (Figure 5). If so, then these links would be broken by dephosphorylation in smooth muscle myosin. As a result of

sequence differences in the ELC N-lobe, we can assume that ELC/RLC contacts are likely to be different in smooth muscle than in molluscan myosins. If those contacts are not extensive, then it would appear that regulation in both types of myosin depends critically on the ability to modulate the stability of key interactions with the RLC C-lobe.

Conclusion

To understand the basis of the on-off triggers in regulated myosins, we have determined structures for Ca^{2+} -bound and Ca^{2+} -free scallop RD reconstituted with complete (non-proteolyzed) light chains. These molluscan structures indicate that, when Ca^{2+} dissociates from the ELC of scallop myosin, a small conformational change near the Ca^{2+} binding site leads to a loss of interactions between the ELC N-lobe and RLC C-lobe. This finding, together with elevated B-factors in nearby HC residues in the absence of Ca^{2+} binding, supports a previous hypothesis³ that the HC of Ca^{2+} -free molluscan myosin becomes significantly more flexible than when Ca^{2+} is bound. Although we have not been able to draw definitive conclusions about the phosphorylation trigger in smooth muscle, it is possible that in both molluscan and smooth muscle myosins the trigger modulates the intrinsic flexibility of the RD and thereby controls the number of conformations available to the myosin head. We propose here that disruption of key links to the RLC C-lobe may be the basis for initiating the off-state in all regulated myosins.

Materials and Methods

Protein preparation and purification

The scallop RD heavy chain fragment was prepared from papain-digested myosin and reconstituted with intact RLC and ELC as previously described²⁶. The D19A ELC mutant was obtained by subcloning into the Nde I and EcoRI sites of pMW172 as described⁹. Purified RD was stored on ice in 5 mM HEPES pH 7, 20 mM NaCl, 2 mM MgCl_2 , 1 mM EGTA, 3 mM NaN_3 , 0.5 $\mu\text{g/ml}$ leupeptin, 0.7 $\mu\text{g/ml}$ pepstatin, and, for the Ca^{2+} -RD, 0.2 mM CaCl_2 . The D19A-RD preparation was dialyzed extensively against large volumes of 1 mM EGTA overnight to remove Ca^{2+} ions.

Crystallization and data collection

Crystals were grown at 4°C in microseeded hanging drops consisting of equal volumes of 16.9 mg/ml protein and precipitant solution (for Ca^{2+} -RD: 150 mM HEPES pH 7.0, 50 mM $(\text{NH}_4)_2\text{SO}_4$, 0.5 mM CaCl_2 , 5 mM MgCl_2 , 5 mM NaN_3 , 16.5% (wt/vol) PEG 4000; for D19A-RD: 100 mM bicine pH 8.3, 45 mM $(\text{NH}_4)_2\text{SO}_4$, 5 mM MgCl_2 , 2.5 mM EGTA, 3.0 mM NaN_3 , 15% (wt/vol) PEG 4000, 4% (vol/vol) PEG 200, 6% (wt/vol) 2,3-butanediol). Crystals were transferred to mother liquor containing 25% (vol/vol) glycerol as a cryoprotectant. They were subsequently flash-cooled in liquid propane, stored as frozen popsicles in liquid N_2 . Crystals for both Ca^{2+} -RD and D19A-RD were triclinic. Data were collected at a temperature of 100 Kelvin at the Brookhaven National Laboratory National Synchrotron Light Source and the Cornell High Energy Synchrotron Source and were processed using HKL-DENZO-SCALEPACK³¹.

Structure determination and refinement

Phases were solved by molecular replacement and rigid body refinement with the program AMoRe³² using a proteolytically-derived scallop Ca^{2+} -RD structure (PDB accession number 1WDC)³ as an initial search model. Stepwise model building and refinement were conducted using the "O" graphics package³³, the Coot graphics package³⁴, XPLOR³⁵, and CNS³⁶ with a bulk solvent correction. Water molecules were added manually in the final stages of refinement.

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Figure 1. Overall Comparison of Structures for Ca^{2+} -RD and D19A-RD

(a) The two structures are shown side by side. The RD consists of a heavy chain (HC, red) and associated light chains: the ELC (green), and the RLC (blue). The light chain helices are labeled A through H, starting from the N-terminus. The four "EF-hand" motif loops of each of the light chains are labeled with Roman numerals. The Ca^{2+} binding site is located in an ELC loop near the ELC/RLC interface. The overall RD conformation is the same in both structures, probably as a result of stabilization from crystal contacts. Inset (right): Schematic diagram showing the entire myosin head with its subdomains. The RD comprises the lever arm, which is a continuation of the converter. (b) A close view of the Ca^{2+} binding site in the Ca^{2+} -RD structure in wall-eyed stereo. As previously reported^{3; 4}, the Ca^{2+} ion is coordinated by seven oxygen ligands, including the carbonyl of Gly23. The electron density (light blue) is from a $2F_o - F_c$ difference map contoured at 1.0 sigma. All residues are highly ordered. (c) The same site as in b for the D19A-RD structure. A $2F_o - F_c$ omit map contoured at 1.0 sigma (light blue) as in b. The Gly23 carbonyl and the side-chain of Asp22 have rotated away from the Ca^{2+} site. We have modeled Asp25 with the side chain facing the Ca^{2+} site, but there is some density for a second conformation pointing away from the site. Arg24 is partially disordered. A water molecule has been modeled at the Ca^{2+} position (see text).

Figure 2. Flexibility of the HC as a Function of Ca^{2+}

The D19A-RD (upper left) and Ca^{2+} -RD structures are shown as a B-factor color ramp (high B-factors, red, and low B-factors, blue) in ribbon diagrams. Main-chain mean B-factors are plotted as a function of residue number for each chain (right and below). Only in the D19A-RD structure, HC B-factors peak near the ELC/RLC interface and at the hook (residues Trp824-Trp826) (red arrows). B-factors spike in the Ca^{2+} binding site only in the absence of Ca^{2+} (green arrows). In the RLC N-lobe, a rise in B-factors is more pronounced for D19A-RD than Ca^{2+} -RD. ELC residue 95 interacts hydrophobically with the Ca^{2+} binding site of a symmetry molecule in the crystal lattice and experiences a B-factor spike only in the absence of Ca^{2+} (black arrow). These results are consistent with the possibility that Ca^{2+} dissociation leads to significantly greater flexibility by the ELC/RLC interface. We cannot currently account for the B-factor rise in the HC hook and RLC N-lobe of the Ca^{2+} -free D19A-RD structure.

Figure 3. Ca²⁺ binding stabilizes HC/RLC/ELC interactions in scallop structures

(a) Shown is a vast hydrogen-bond and covalent bond network in the Ca²⁺-RD structure extending from the Ca²⁺ binding site to Lys149 on the RLC N-lobe. (b) Part of this network forms a meshwork that stabilizes the coordination of the Ca²⁺ ion, shown here schematically. Solid gray lines indicate main-chain interactions and wavy lines indicate side-chain interactions. (c) The same meshwork as in (b) in the absence of Ca²⁺. Notice that when the Gly23 carbonyl rotates away from the Ca²⁺ binding site, four hydrogen bonds from this meshwork are abolished.

Figure 4. Location of the phosphate binding site in smooth muscle myosin

The phosphorylatable serine in smooth muscle myosin is equivalent to scallop residue S6 (arrow). In smooth muscle, the RLC N-terminus is longer and contains a kinase binding site. Notice that residue S6 is located in close proximity to helix A. Phosphorylation could promote links between this region and helices E or H. Consistent with this possibility, two different secondary structure prediction programs (Jpred3, <http://www.compbio.dundee.ac.uk/~www-jpred/>; PredictProtein, <http://www.predictprotein.org/>) predict that at least part of this loop between S6 and helix A has a high propensity to form a helix. Recent experimental evidence and computational studies support the view that this region extends helix A upon phosphorylation³⁷.

Figure 5. Scallop and Squid RLC

Scallop (color) and squid (gray tones, PDB Code 2OVK30) are superposed (superposition based on scallop HC residues 807–818 and equivalent squid HC residues 809–820). The scallop structure is shown oriented as in Figure 4. In the squid crystal structure, the RLC N-lobe is rotated closer to the C-lobe, bringing helix A and the RLC N-terminus within reach of helices E and H. RLC residue C α distances are shown in Å for (1) scallop Asp84 - Lys149 (magenta), scallop Pro13 - Asp84 (tan), and squid Arg13 - Glu84 (black).

Table 1

Some Data Collection and Refinement Statistics.*

	<u>Ca²⁺-RD</u>	<u>D19A-RD</u>
<u>PDB ID Code</u>	XXXX	3JTD
<u>Data Collection</u>		
Resolution Range (Å)	50.0 – 2.10	34.9 – 2.57
R _{sym} , % (All/Outer Shell, 2.1 Å, below)	4.7/12.9	6.4/13
Unique Reflections/Multiplicity	25062/1.8	15190/2.5
Average I/σ	9.0	12.3
Completeness (%):		
All Data	95.1	97.5
2.69 – 2.57 Å Shell	95.8	96.2
2.13 – 2.10 Å Shell	94.4	-
<u>Refinement</u>		
Sigma Cutoff	0.0	0.0
Completeness of Used Reflections (%)	92.4	90.5
R-factor/R _{free} (%)	20.2/23.5	23.3/24.8
Cross-validated Coordinate Error (Å) ^{37; 38; 39,;}	0.30	0.46
No. of Protein/Water Atoms	3017/269	2957/58
No. of Cations	2	1
Mean B-factor	33.2	48.7
Protein	32.6	48.8
Solvent	40.2	40.8
Cations	37.4	35.3
RMS Bond Lengths (Å)/Bond Angles(°)	0.010/1.67	0.010/1.70
<u>Ramachandran Regions</u>		
Most favored	90.6%	93.3%
Additional Allowed	9.4%	6.2%
Generous or disallowed	0%	0.5%

* The cell parameters of these crystal structures are: Ca²⁺-RD: P1, a=46.7, b=52.2, c=55.3 Å, α=65.3°, β=66.4°, γ=70.2°; D19A-RD: P1, a=42.0, b=52.8, c=58.5 Å, α=113.4°, β=91.6°, γ=100.6°